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(54) Title: **COMBINATION OF AN ANTI-EP-CAM ANTIBODY WITH A CHEMOTHERAPEUTIC AGENT**

(57) Abstract: A combination of an anti-Ep-CAM antibody with a chemotherapeutic agent that is capable of arresting Ep-CAM antigen expressing cells in S or G₂/M.

COMBINATION OF AN ANTI-EP-CAM ANTIBODY WITH A CHEMOTHERAPEUTIC AGENT

5 This present invention relates to the combination of antibodies that specifically bind to the EP-CAM antigen with chemotherapeutic agents that affect cell growth by blocking progression of the cell cycle in G₂/M and their use in therapy of cancers which express the antigen.

10 The conventional therapeutic approaches to cancer include surgery, radiotherapy and chemotherapy in various combinations; however, response rates have not improved significantly in the last 20 years. The major limitation of chemotherapy and radiotherapy is the non-selective targeting of both normal and tumour cells that results in toxic side effects. In the search for less toxic and more specific treatment alternatives, various types of immunotherapy have been investigated. Among these modalities, strategies based on monoclonal antibodies have been applied to a broad spectrum of malignancies (Riethmüller et al. Curr Opin Immun 1992, 4, 647-655 and Riethmüller et al. Curr Opin Immunol 1993, 5, 732-739). The utility of monoclonal antibodies is based upon their clonal antigen specificity, i.e. molecular recognition of specific epitopes which may comprise an antigen and to bind to these antigens with high affinity. 15 Monoclonal antibodies can bind to antigens expressed uniquely or preferentially on the surface of malignant cells, and hence can be used to specifically target and destroy tumour cells. Antibodies may be constructed as delivery vehicles for drugs or DNA, or as conjugates with radionuclides. Binding of naked antibody to target cells may also activate innate antitumour immune functions such as antibody-dependent cell-mediated cytotoxicity (ADCC) and complement-mediated cytotoxicity (CMC), either of which may result in lysis or phagocytosis of the targeted cell. Both ADCC and CMC are antibody-dose-related immune functions and it is therefore desirable to get as much antibody bound to target cells as possible. One way of achieving this objective is to increase the level of expression of the relevant antigen which may effectively increase antibody functions such as, for example, ADCC of the target cells by virtue of getting 20 more antibody bound to the cells (Fogler et al. Cancer Research 48 : 6303-6308 (1988)). 30

One antigen of importance in cancer therapy is the Ep-CAM antigen (human pan-carcinoma antigen). This antigen is a transmembrane glycoprotein which has been reported to function as a cell adhesion molecule (Litvinow et al. J. Cell Biology 125: 437-446, 1994) and is also known as the 17-1A antigen, 40kD antigen, EGP40, GA733-2, KSA and ESA but may be known by other names or descriptions in the literature as well. It is expressed on the baso-lateral surface of a majority of simple cuboidal or columnar, pseudo stratified columnar and transitional epithelia and at generally higher levels in tumour cells. Epithelial cells are known to be the most important cell type in the development of human malignancies. Thus more than 90% of all malignant tumours are carcinomas, and therefore of epithelial origin (Acta Anatomica; 156 (3); 217-226 (1996)). Although the Ep-CAM antigen is expressed on most tumour cells of epithelial origin there are examples of cells of epithelial origin that do not express Ep-CAM such as adult epithelial tissues, epidermal adult keratinocytes, gastric parietal cells, thymic cortical epithelium, myoepithelial cells and hepatocytes. The phenotype of a malignant cell plays an important role in the efficacy of monoclonal antibodies. While tumour specific antigens have proven to be elusive, differences in expression of the antigens between normal cells and tumour cells have provided a means to target antibodies to tumours. It would be clinically advantageous to improve on these differences by enhancement of antigen homogeneity and density of expression on tumour cells.

Interferons are well-known to alter cell phenotypes by increasing expression of tumour antigens as well as many normal antigens, e.g. Class I HLA. For example, human recombinant interferon- α and interferon- γ can increase the expression of human tumour antigens TAG-72 and CEA (Greiner et al. Cancer Res 44:3208-3214 (1984)). Interferon exposure induced a more homogeneous CEA-positive tumour cell population which shed more CEA from the cells surface, which was confirmed by *in vivo* studies with human carcinoma xenografts in athymic mice. Treatment with interferon- γ enhanced TAG-72 and CEA expression on ovarian or gastrointestinal tumour cells in patients' malignant ascites (Greiner et al. J Clin Oncol 10:735-746 (1992)). The effects of interferons on cells are myriad and range from direct cytotoxicity to immunopotentialiation to antiproliferative activity. None of the effects of interferons

on antigen expression have been directly ascribed to interference with cell cycle progression.

Briefly, cell cycle progression refers to the sequence of events between one mitotic division and another in a cell. A quiescent resting phase (G_0) is followed by a growth phase (G_1), then by a DNA synthesis phase (S). A second growth phase of cell enlargement (G_2) and DNA replication (M phase) is followed by division of the cell into two progeny cells. Any interference with the cell machinery may inhibit all cycle progression at any phase of the cell cycle. For example, specific chemotherapeutic agents may block progression in either G_2 or M or in both G_2 and M (G_2/M). In other words exposure to certain drugs e.g. chemotherapeutic agents will for example, arrest individual cells in G_2 and/or M until eventually most, or all of the cells in a population become arrested in G_2 and/or M (G_2/M). In HeLa cells, for example, the G_1 , S, G_2 and M phase take 8.2, 6.2, 4.6 and 0.6 hours, respectively. The period between mitoses is called interphase. Cells may have different doubling times, depending on their developmental stage or tissue type. The variation in doubling times is usually a function of the time spent in G_1 (A Dictionary of Genetics, 5th edition, RC King and WD Stansfield, Oxford University Press, 1997).

While a few proteins have been identified as produced solely at certain phases of the cell cycle, and therefore can serve as markers of cell cycle status, most others are produced across the cell cycle but at higher or lower levels at certain points. Variation of antigen density across the cell cycle is typical for the sarcoma antigens p102 and p200 (Song S, Anticancer Research 16(3A) : 1171-5 (1996)), the leukaemia/lymphoma-associated antigen JD118 (Czuczman et al. Cancer Immunology, Immunotherapy 36(6):387-96 (1993)), and the gastric tumour antigen PC1 (Wei et al., J of Oncology 9(3) : 179-82 (1987)). A few tumour antigens have been reported to be cell-cycle independent, e.g. liver metastases 3H4 (Wulf et al., J. Cancer Research and Clinical Oncology 122(8) : 476-82 (1996)) and small cell lung cancer antigens (Fargion et al., Cancer Research 46 : 2633-2638 (1986)).

Surprisingly, it has been found that pre-treatment with a drug, for example a chemotherapeutic agent known to block cell cycle progression at S and/or G_2/M results in a significant increase in the density of the Ep-CAM antigen population

and thus in greater targeting of anti-Ep-CAM antibodies to Ep-CAM expressing tumours. In lytic antibodies this translates into an increased susceptibility to antibody-dependent cytotoxicity. This perturbation of tumour cell phenotype has a significant impact on the biological effectiveness of therapeutic antibodies, and provides synergistic benefit to standard chemotherapeutic regimens. Furthermore, this increase in Ep-CAM antigen expression appears to be tumour specific. In other words, pre-treatment with chemotherapeutic agents that block the cell cycle at S and/or G₂/M does not seem to affect Ep-CAM antigen expression in non-tumour cells.

Accordingly, the present invention provides a combination of an Ep-CAM antibody and a chemotherapeutic agent that is capable of arresting Ep-CAM antigen expressing cells in S or G₂/M, preferably in G₂/M.

Examples of anti-Ep-CAM antibodies are ING1 (Colcher et al., Proc. Natl. Acad. Sci. USA, 78 (5), 3199 to 3203 (1981) and Laio et al, Human Antibody Hybridomas 1(2), 66 -76 (1990)); 17-1A e.g. Panorex (Herlyn et al, Proc. Natl. Acad. Sci. USA 76 : 1438 - 1452 (1979) and Herlyn et al, Hybridoma 1985; 5 (suppl. 1) S3 to S10); and NR-LU-10 (Okabe et al, Cancer Research, 44, 5273 to 5278 (1984)).

Panorex (Adjuqual®) is a 17.1A mouse monoclonal antibody. It is marketed by Glaxo Wellcome in Germany for the post-operative adjuvant therapy of colorectal cancer.

An example of an anti-Ep-CAM antibody is one with the variable light chain cDNA sequence as set out in Figure 15 and the heavy chain cDNA sequence as set out in Figure 16. (known as humanised 323/A3/IgG₁). Two further preferred examples of anti- Ep-CAM antibodies are those with the variable light chain cDNA sequence as set out in Figures 15 and heavy chain cDNA sequences as set out in Figures 17 or 18 respectively (known as humanised 323/A3 IgG₄ and IgG₂cys respectively).

A preferred example of an anti-Ep-CAM antibody is 17.1A, most preferably Panorex.

Specific anti-Ep-CAM antibodies can be given on their own or in combination with other anti-Ep-CAM antibodies. Examples of such anti-Ep-CAM antibody combinations are an anti-Ep-CAM antibody with the variable light chain cDNA sequence as set out in Figure 15 and the heavy chain cDNA sequence as set out in Figure 16 in combination with ING1. Thus throughout the specification reference to an anti-Ep-CAM antibody includes antibody combinations of various anti-Ep-CAM antibodies, preferably non-competing anti-Ep-CAM antibodies targeting different epitopes on the same Ep-CAM antigen.

Examples of chemotherapeutic agents which are capable of arresting Ep-CAM antigen expressing cells in G₂/M are vinorelbine, cisplatin, mytomyacin, paclitaxel, carboplatin, oxaliplatin and CPT-II (camptothecin).

Vinorelbine tartrate is a semisynthetic vinca alkaloid with the chemical name 3',4'-didehydro -4'-deoxy-C'-norvincal leukoblastine [R-(R*,R*)-2,3-dihydroxybutanedioate (1:2)(salt)]. Vinorelbine tartrate is used in combination with other chemotherapy agents such as cisplatin or as a single agent in the treatment of various solid tumours particularly non-small cell lung, advanced breast, and hormone refractory prostate cancers. The brand name Navelbine® is used in North America and Europe. Navelbine® is administered intravenously as a single-agent or in combination therapy typically at doses of 20-30 mg/m² on a weekly basis. An oral formulation of vinorelbine is in clinical development.

Cisplatin has the chemical name cis-diamminedichloroplatinum. Cisplatin is used in the treatment of metastatic testicular tumours as a combination therapy, as single and combination therapy in metastatic ovarian tumours, as well as a single agent in advanced bladder cancer. Cisplatin is manufactured by Bristol-Myers Squibb under the brand names of Platinol® and Platinol-AQ®. Cisplatin is also used in the following types of cancer, typically in combination therapy: non-small cell and small cell lung cancers, head and neck, endometrial, cervical, and non-Hodgkin's lymphoma. Cisplatin is typically administered intravenously in doses ranging from 15-150 mg/m² once every 3 to 4 weeks, or daily for 5 days repeated every 3 or 4 weeks. However, higher and more frequent doses are occasionally administered and the route of administration could be different than intravenous, such as intra-arterial or intraperitoneal.

Carboplatin has the chemical name platinum, diammine [1,1-cyclobutane-dicarboxylato(2)-0,0']-(SP-4-2). Carboplatin is usually administered in combination with other cytotoxics such as paclitaxel and etoposide. It is used in the treatment of advanced ovarian cancer, non-small cell lung cancer as well as in many of the same types of cancer as cisplatin is used. The brand name of carboplatin manufactured by Bristol-Myers Squibb is Paraplatin®. Carboplatin is typically administered intravenously at 300 - 400 mg/m², or to a target area under the drug concentration versus time curve (AUC) of 4-6 mg/ml-min using the patient's estimated glomerular filtration rate (GFR). Higher doses up to around 1600 mg/m² divided over several, usually five, days may also be administered.

Paclitaxel has the chemical name 5 β , 20 epoxy-1,2 α ,4,7 β ,10 β ,13 α -hexahydroxytax-11-en-9-one 4,10-diacetate 2-benzoate 13-ester with (2R, 3S)-N-benzoyl-3-phenylisoserine. Paclitaxel is manufactured by Bristol-Myers Squibb as Taxol®. It is used to treat a variety of carcinomas including ovarian, breast, non-small cell lung, head and neck. Typical doses include 135-175 mg/m² as either a 3 or 24 hour intravenous infusion given every 3 or 4 weeks. Higher doses up to around 300 mg/m² have also been administered.

Besides the active ingredient, the drug products provided by manufacturers typically contain a diluent such as sterile water, dextrose 5% in water or 0.9% sodium chloride in water with additional excipients such as Cremophor vehicle added to make for example, paclitaxel soluble.

More detailed information on treatment regimes, dosages and compositions etc can be obtained from standard reference books such as: Martindale, The Extra Pharmacopoeia, 31st edition, edited by JEF Reynolds, London, Royal Pharmaceutical Society, 1996 and the Physicians Desk reference, 49th Edition, 1995, Medical Economics Data Production Company, Montvale.

Other chemotherapeutic agents that may cause cells to accumulate in G₂ /M include anthracyclines e.g. doxorubicin and aclarubicin; carmustine (BCNU), camptothecin, 9-nitro-camptothecin, cyclophosphamide and its derivatives,

docetaxel, etoposide, Razoxane (ICRF-187), alkyllyso-phospholipids e.g. ilmofosine; methotrexate, MST-16, taxanes, vinblastine, vincristine and teniposide (VM-26) (again see Martindale, The Extra Pharmacopoeia, 31st edition, edited by JEF Reynolds, London, Royal Pharmaceutical Society, 1996,) and flavonoids e.g. apigenin and genistein (see The Merck Index, 12th edition, Merck Research Laboratories, Merck and Co Inc, 1996). In addition, adozelesin (a class of pyrazole compounds) (Cancer Research 1992, October 15; 52 (2) : 5687 to 5692)), Bistratene A (Mutation Research 1996, March 1; 367 (3) : 169 to 175), cyclophosphamide (Cancer Chemotherapy & Pharmacology 1994; 33(5) : 399 to 409), imidazoarcridinone, mefloquine (Experimental Cell Biology 1986; 54 (3) : 138 to 148 and International Journal of Cancer 1995, Jul 17; 62 (2) : 170 to 175), merbarone (Environmental & Molecular Mutagenesis 1997; 29 (1) : 16 to 27 and Cancer Research 1995, Apr 1; 55 (7) : 1509 to 1516) and oracin (FEBS Letters 1997, Jan 2; 400 (1) : 127 to 130) are also believed to cause cells to accumulate in G₂/M generally all topo II inhibitors, e.g. to topotecan (abpi, 1998-1999), all vinca derivatives and all DNA damaging agents including radiation are also believed to arrest cells in G₂/M.

Moreover, 5FU has been reported to arrest cells in G₂/M (Oncology Research 1994; 6(7):303-309) and it is therefore believed that 5FU and compounds similar to 5FU such as UFT, methotrexate, capecitabine and Gemcitabine will increase Ep-Cam expression in some tissues. Similarly, tomudex (Raloxifen) which is known to arrest cells in the S phase is believed to increase Ep-Cam expression.

The term "chemotherapeutic agent" throughout the specification is therefore not limited to cytotoxic therapy, but also encompasses cytostatic therapy and any other drugs capable of stopping cells in G₂/M. It should be further noted that radiotherapy is capable of arresting cells in G₂/M and that throughout the specification the term chemotherapeutic can therefore be substituted with "radiotherapy".

Throughout the specification reference to a chemotherapeutic agent includes combinations of one or more specific chemotherapeutic agents which arrest Ep-CAM expressing tumour cells in G₂/M. Examples of typical combinations are vinorelbine with cisplatin and paclitaxel with carboplatin; oxaliplatin with 5FU;

cyclophosphamide with methotrexate and 5FU; cyclophosphamide with doxorubicin and 5FU.

5 While it is possible for the chemotherapeutic agent to be administered alone it is preferable to present it as a pharmaceutical composition comprising an active ingredient, as defined above, together with an acceptable carrier therefor. Each carrier must be "acceptable" in the sense of being compatible with the other ingredients of the composition and not injurious to the recipient.

10 Preferred combinations of an Ep-Cam antibody and a chemotherapeutic agent(s) that are capable of arresting Ep-CAM antigen expressing cells in S or G₂/M are: Panorex in combination with any of the following chemotherapeutic agents: UFT, Capecitabine, CPT-II, Oxaliplatin, 5FU, 5FU continuous infusion, Paclitaxel, Docetaxel, Cyclophosphamide, Methotrexate, Doxorubicin, Navelbine
15 (iv and oral), Epirubicin, Mitoxantrone, Raloxifen, Cisplatin, Mitomycin, Carboplatinum, Gemcitabine, Etoposide and Topotecan.

Particularly preferred combinations are Panorex with CPT-II, 5FU (continuous infusion), Oxaliplatin, Capecitabine, UFT and Tomudex (Raloxifen).

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These Panorex combinations are useful in the treatment of cancer, particularly in the treatment of colorectal cancer, breast cancer, gastric cancer, prostate cancer and non-small-cell lung cancer.

25 Specifically, the following combinations are particularly preferred for colorectal cancer: Panorex in combination with: UFT (optionally with Leucovorin); Capecitabine; Oxaliplatin (optionally with 5FU); CPT-II, 5FU (optionally with Eniluracil or Levamisole or Leucovorin); 5FU protected continuous infusion; and Mitomycin.

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Preferred combinations for the treatment of breast cancer are: Panorex in combination with Paclitaxel; Docetaxel; Cyclophosphamide (optionally with 5FU and either Methotrexate or Doxorubicin); Navelbine (iv and/or oral); Doxorubicine; Epirubicin; Mitoxantrone; and Raloxifen.

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Preferred combinations for the treatment of gastric cancer are: Panorex in combination with Cisplatin; 5FU; Mitomycin; and Carboplatinum.

5 A preferred combination for the treatment of prostatic cancer is: Panorex in combination with Mitoxantrone.

Preferred combinations for the treatment of non-small-cell lung cancer are: Panorex in combination with: Navelbine; Cisplatin; Carboplatin; Paclitaxel; Docetaxel; Gemcitabine; Topotecan; and Etoposide.

10 Information regarding dosing of Panorex and the above chemotherapeutic agents given in combination with Panorex can be found in standard reference books such as ABPI, Compendium of Data Sheets and Summaries of Product Characteristics, Datapharm Publications Limited, 1998-1999.

15 The compositions include those suitable for oral, rectal, nasal, topical (including buccal and sublingual), vaginal, parenteral (including subcutaneous, intramuscular, intravenous and intradermal) or transdermal administration. The compositions may conveniently be presented in unit dosage form and may be
20 prepared by any methods well known in the art of pharmacy. Such methods include the step of bringing into association the active ingredient with the carrier which constitutes one or more accessory ingredients. In general, the compositions are prepared by uniformly and intimately bringing into association the active ingredient with liquid carriers or finely divided solid carriers or both,
25 and then if necessary shaping the product.

Compositions of the chemotherapeutic agent suitable for oral administration may be presented as discrete units such as capsules, cachets or tablets each containing a predetermined amount of the active ingredient; as a powder or
30 granules; as a solution or suspension in an aqueous or non-aqueous liquid; or as an oil-in-water liquid emulsion or a water-in-oil liquid emulsion. The active ingredient may also be presented as a bolus, electuary or paste.

35 A tablet may be made by compression or moulding, optionally with one or more accessory ingredients. Compressed tablets may be prepared by compressing in

a suitable machine the active ingredient in a free-flowing form such as a powder or granules, optionally mixed with a binder (e.g. povidone, gelatin, hydroxypropylmethyl cellulose), lubricants, inert diluent, preservative, disintegrant (eg. sodium starch glycollate, cross-linked povidone, cross-linked sodium carboxymethyl cellulose) surface-active or dispersing agent. Moulded tablets may be made by moulding in a suitable machine a mixture of the powdered compound moistened with an inert liquid diluent. The tablets may optionally be coated or scored and may be formulated so as to provide slow or controlled release of the active ingredient therein using, for example, hydroxypropylmethyl cellulose in varying proportions to provide the desired release profile. Tablets may optionally be provided with an enteric coating to provide release in parts of the gut other than the stomach.

Compositions suitable for oral use as described above may also include buffering agents designed to neutralise stomach acidity. Such buffers may be chosen from a variety of organic or inorganic agents such as weak acids or bases admixed with their conjugated salts.

Composition suitable for topical administration in the mouth include lozenges comprising the active ingredient in a flavoured basis, usually sucrose and acacia or tragacanth; pastilles comprising the active ingredient in an inert basis such as gelatine and glycerin, or sucrose and acacia and mouthwashes comprising the active ingredient in a suitable carrier.

Compositions for rectal administration may be presented as a suppository with suitable base comprising for example cocoa butter or a salicylate.

Compositions suitable for vaginal administration may be presented as pessaries, tampons, creams, gels, pastes, foams or spray formulations containing in addition to the active ingredient such carriers as are known in the art to be appropriate.

Compositions suitable for parenteral administration include aqueous and non-aqueous isotonic sterile injection solutions which may contain anti-oxidants, buffers, bacteriostats and solutes which render the compositions isotonic with

the blood of the intended recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents and thickening agents, such as liposomes or other microparticulate systems which are designed to target the compounds to blood components or one or more organs. The compositions may be presented in unit-dose or multi-dose sealed containers, for example, ampoules and vials, and may be stored in a freeze-dried(lyophilized) condition requiring only the addition of sterile liquid carrier, for example water for injections, immediately prior to use. Extemporaneous injection solutions and suspensions may be prepared from sterile powders, granules and tablets of the kind previously described.

Compositions suitable for transdermal administration may be presented as discrete patches adapted to remain in intimate contact with the epidermis of the recipient for a prolonged period of time. Such patches suitably contain the active ingredient as an optionally buffered, aqueous solution of, for example, 0.1 to 0.2M concentration with respect to said compound. As one particular possibility, the active ingredient may be delivered from the patch by iontophoresis as generally described in Pharmaceutical Research, 3 (6),318 (1986).

It should be understood that in addition to the ingredients particularly mentioned above the compositions in question, for example, those suitable for oral administration may include such further agents as sweeteners, thickeners and flavouring agents.

The dosage range of the chemotherapeutic agent to be co-administered with the antibody may typically be between about 1 to 1000 mg/m² (based on patient body surface area) or about 2 - 30 mg /kg (based on patient body weight), depending on the chemotherapeutic agent(s) used. Thus, for example, vinorelbine (navelbine) would typically be administered at a dosage of about 20 to 30 mg/m², cisplatin at about 15 to 100mg/m² carboplatin at about 300 to 600 mg/m² and paclitaxel at about 100 to 300 mg/m², preferably around 135 to 175 mg/m². Another way of expressing dosage is by their AUC value. For example carboplatin would typically be administered at a dose calculated as AUC = 4 to 6mg/ml-min. Generally, the doses of chemotherapeutic agents are lower when given in combination with another chemotherapeutic agent and/or antibody than if given on their own as the single chemotherapeutic agent. The doses of

chemotherapeutic agents that will be co-administered with anti Ep-CAM antibody(ies) will likely be the standard doses for the type of carcinoma treated or lower doses. In general the highest tolerated doses of the chemotherapeutic agents are administered either alone or in combination.

5

The anti-Ep-CAM antibodies of the present invention preferably have the structure of a natural antibody or a fragment thereof. Antibodies typically comprise two heavy chains linked together by disulphide bonds and two light chains. Each light chain is linked to a respective heavy chain by disulphide bonds. Each heavy chain has at one end a variable domain followed by a number of constant domains. Each light chain has a variable domain at one end and a constant domain at its other end. The light chain variable domain is aligned with the variable domain of the heavy chain. The light chain constant domain is aligned with the first constant domain of the heavy chain. The constant domains in the light and heavy chains are not involved directly in binding the antibody to antigen.

The variable domains of each pair of light and heavy chains form the antigen binding site. The domains on the light and heavy chains have the same general structure and each domain comprises a framework of four regions, whose sequences are relatively conserved, connected by three complementarity determining regions (CDRs). The four framework regions largely adopt a beta-sheet conformation and the CDRs form loops connecting, and in some cases forming part of the beta-sheet structure. The CDRs are held in close proximity by the framework regions and with the CDRs from the other domain, contribute to the formation of the antigen binding site, which in the case of the present invention is the formation of an anti-Ep-CAM binding site. CDRs and framework regions of antibodies may be determined by reference to Kabat *et al* ("Sequences of proteins of immunological interest" US Dept. of Health and Human Services, US Government Printing Office, 1987).

The preparation of an antibody in which the CDRs are derived from a different species than the framework of the antibody's variable domains is disclosed in EP-A-0239400. The CDR's may be derived from a rodent or primate monoclonal antibody. The framework of the variable domains and the constant

domains of such altered antibodies are usually derived from a human antibody. Such a humanised antibody should not elicit as great an immune response when administered to a human compared to the immune response mounted by a human against a wholly foreign antibody such as one derived from a rodent.

5

The antibody preferably has the structure of a natural antibody or a fragment thereof. Throughout the specification reference to antibody therefore comprises not only a complete antibody but also fragments such as a (Fab')₂ fragment, a Fab fragment, a light chain dimer or a heavy chain dimer. The antibody may be an IgG such as IgG₁, IgG₂, IgG₃ or IgG₄; or IgM, IgA, IgE or IgD or a modified variant thereof, including those that may be conjugated to other molecules such as radionuclides, enzymes etc. Typically, the constant region is selected according to the functionality required. Normally an IgG1 will demonstrate lytic ability through binding to complement and will mediate ADCC (antibody dependent cell cytotoxicity). An IgG₄ antibody will be preferred if a non-cytotoxic antibody is required. Antibodies according to the present invention also include bispecific antibodies such as, for example, the 17-1A antibody disclosed in Mack et al, The Journal of Immunology, 1997, 158 : 3965 -3970. Antibodies of the present invention may be murine, chimaeric or humanised with the preferred antibody being humanised antibody.

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There are four general steps to humanise a monoclonal antibody. These are :

- (1) determining the nucleotide and predicted amino acid sequence of the starting antibody light and heavy variable domains ;
- (2) designing the humanised antibody, i.e. deciding which antibody framework region to use during the humanising process ;
- (3) the actual humanising methodologies/techniques; and
- (4) the transfection and expression of the humanised antibody.

25

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More specifically,

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Step 1 : Determining the nucleotide and predicted amino acid sequence of the antibody light and heavy chain variable domains

5 To humanise an antibody only the amino acid sequence of the antibody's heavy and light chain variable domains needs to be known. The sequence of the constant domains is irrelevant because these do not contribute to the reshaping strategy. The simplest method of determining an antibody variable domain amino acid sequence is from cloned cDNA encoding the heavy and light variable domain.

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There are two general methods for cloning a given antibody's heavy and light chain variable domain cDNAs: (1) via a conventional cDNA library, or (2) via the polymerase chain reaction (PCR). Both of these methods are widely known. Given the nucleotide sequence of the cDNAs, it is a simple matter to translate this information into the predicted amino acid sequence of the antibody variable domains.

15

Step 2 : Designing the humanised antibody

20 There are several factors to consider in deciding which human antibody sequence to use during the humanisation. The humanisation of light and heavy chains are considered independently of one another, but the reasoning is basically similar for each.

25 This selection process is based on the following rationale : a given antibody's antigen specificity and affinity is primarily determined by the amino acid sequence of the variable region CDRs. Variable domain framework residues have little or no direct contribution. The primary function of the framework regions is to hold the CDRs in their proper spatial orientation to recognise the antigen. Thus the substitution of rodent CDRs into a human variable domain framework is most likely to result in retention of their correct spatial orientation if the human variable domain framework is highly homologous to the rodent variable domain from which they originated. A human variable domain should preferably be chosen therefore that is highly homologous to the rodent variable domain(s).

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A suitable human antibody variable domain sequence can be selected as follows :

- 5 1. Using a computer program, search all available protein (and DNA) databases for those human antibody variable domain sequences that are most homologous to the rodent antibody variable domains. The output of a suitable program is a list of sequences most homologous to the rodent antibody, the percent homology to each sequence, and an alignment of each
10 sequence to the rodent sequence. This is done independently for both the heavy and light chain variable domain sequences. The above analyses are more easily accomplished if only human immunoglobulin sequences are included.
- 15 2. List the human antibody variable domain sequences and compare for homology. Primarily the comparison is performed on lengths of CDRs, except CDR 3 of the heavy chain which is quite variable. Human heavy chains and Kappa and Lambda light chains are divided into subgroups; Heavy chain 3
20 subgroups, Kappa chain 4 subgroups, Lambda chain 6 subgroups. The CDR sizes within each subgroup are similar but vary between subgroups. It is usually possible to match a rodent antibody CDR to one of the human subgroups as a first approximation of homology. Antibodies bearing CDRs of similar length are then compared for amino acid sequence homology, especially within the CDRs, but also in the surrounding framework regions.
25 The human variable domain which is most homologous is chosen as the framework for humanisation.

Step 3 : The actual humanising methodologies/techniques

- 30 An antibody may be humanised by grafting the desired CDRs onto a human framework according to EP-A- 0239400.(see also P.T. Jones et al, Nature 321:522 (1986); L. Reichman et al, Nature 332 :323(1988); Verhoeyen M. et al, Science 239:1534 (1988) and J. Ellis et al, The Journal of Immunology, 155 :925-937(1995)). A DNA sequence encoding the desired reshaped antibody
35 can therefore be made beginning with the human DNA whose CDRs it is wished

to reshape. The rodent variable domain amino acid sequence containing the desired CDRs is compared to that of the chosen human antibody variable domain sequence. The residues in the human variable domain are marked that need to be changed to the corresponding residue in the rodent to make the human variable region incorporate the rodent CDRs. There may also be residues that need substituting in, adding to or deleting from the human sequence.

Oligonucleotides are synthesised that can be used to mutagenise the human variable domain framework to contain the desired residues. Those oligonucleotides can be of any convenient size. One is normally only limited in length by the capabilities of the particular synthesiser one has available. The method of oligonucleotide-directed in vitro mutagenesis is well known.

Alternatively humanisation may be achieved using the recombinant polymerase chain reaction (PCR) methodology of WO92/07075. Using this methodology, a CDR may be spliced between the framework regions of a human antibody.

In general, the technique of WO92/07075 can be performed using a template comprising two human framework regions, AB and CD and between them, the CDR which is to be replaced by a donor CDR. Primers A and B are used to amplify the framework region AB, and primers C and D used to amplify the framework region CD. However, the primers B and C each also contain, at their 5' ends, an additional sequence corresponding to all or at least part of the donor CDR sequence. Primers B and C overlap by a length sufficient to permit annealing of their 5' ends to each other under conditions which allow a PCR to be performed. Thus, the amplified regions AB and CD may undergo gene splicing by overlap extension to produce the humanised product in a single reaction.

Step 4 : The transfection and expression of the reshaped antibody

Following the mutagenesis reactions to reshape the antibody, the mutagenised DNAs can be linked to an appropriate DNA encoding a light or heavy chain constant region, cloned into an expression vector, and transfected into host

cells, preferably mammalian cells. These steps can be carried out in routine fashion. A reshaped antibody may therefore be prepared by a process comprising :

- 5 (a) preparing a first replicable expression vector including a suitable promoter operably linked to a DNA sequence which encodes at least a variable domain of an Ig heavy or light chain, the variable domain comprising framework regions from a human antibody and the CDRs required for the humanised antibody of the invention.
 - 10 (b) preparing a second replicable expression vector including a suitable promoter operably linked to a DNA sequence which encodes at least the variable domain of a complementary Ig light or heavy chain respectively;
 - 15 (c) transforming a cell line with the first or both prepared vectors; and
 - d) culturing said transformed cell line to produce said altered antibody.
- 20 Preferably the DNA sequence in step (a) encodes both the variable domain and the or each constant domain of the human antibody chain. The humanised antibody can be recovered and purified. The cell line which is transformed to produce the altered antibody may be Chinese Hamster Ovary (CHO) cell line or an immortalised mammalian cell line, which is advantageously of lymphoid
- 25 origin, such as a myeloma, hybridoma, trioma or quadroma cell line. The cell line may also comprise a normal lymphoid cell, such as a B-cell, which has been immortalised by transformation with a virus, such as the Epstein-Barr virus. Most preferably, the immortalised cell line is a myeloma cell line or a derivative thereof. The expression system of choice is the glutamine synthetase
- 30 expression system described in WO87/00462 (see also P.E. Stephens et al, Nucleic Acid Res. 17:7110 (1989) and C.R. Bebbington et al, Bio/Technology 10:169 (1992)).

35 Although the cell line used to produce the humanised antibody is preferably a mammalian cell line, any other suitable cell line, such as a bacterial cell line or a

yeast cell line, may alternatively be used. For single antibody chains, it is envisaged that E. coli - derived bacterial strains could be used. The antibody obtained is checked for functionality. If functionality is lost, it is necessary to return to step (2) and alter the framework of the antibody.

5

Once expressed, the whole antibodies, their dimers, individual light and heavy chains, or other immunoglobulin forms of the present invention can be purified according to standard procedures of the art, including ammonium sulfate precipitation, affinity columns, column chromatography, gel electrophoresis and the like (see generally Scopes, R, Protein Purification, Springer-Verlag, N.Y. (1982)). Substantially pure immunoglobulins of at least about 90 to 95% homogeneity are preferred and 98 to 99% or more homogeneity most preferred, for pharmaceutical uses. Once purified, partially or to homogeneity as desired, an antibody may then be used therapeutically.

15

Antibodies are typically provided as a pharmaceutical composition comprising a pharmaceutically acceptable carrier or diluent and, as active ingredient, an antibody according to the invention. The antibody and pharmaceutical compositions thereof are particularly useful for parenteral administration i.e. subcutaneously, intramuscularly or intravenously.

20

The compositions for parenteral administration will commonly comprise a solution of the antibody or a cocktail thereof dissolved in an acceptable carrier, preferably an aqueous carrier. A variety of aqueous carriers can be used, eg. sterile water for injection, 0.9% sodium chloride in water, 5% dextrose in water and Lactated Ringers solution. These solutions are sterile and generally free of particulate matter. These compositions may be sterilised by conventional, well known sterilisation techniques. The compositions may contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions such as pH adjusting and buffering agents, toxicity adjustment agents and the like, for example sodium acetate, sodium chloride, potassium chloride, calcium chloride, sodium lactate, etc. The concentration of antibody in these formulations can vary widely, for example from less than about 0.5%, usually at or at least about 1% to as much as 15 or 20% by weight and will be selected

25

30

primarily based on fluid volumes, viscosities, etc. in accordance with particular mode of administration selected.

5 Thus, a typical pharmaceutical composition for intramuscular injection could be made to contain 1 ml sterile buffered water, and 50 mg of antibody. A typical composition for intravenous infusion could be made up to contain 250 ml of sterile Ringers solution and 150mg of antibody. Actual methods for preparing parenterally administrable compositions will be known or apparent to those skilled in the art, particularly, those trained in the preparation of parenteral products and are described in more detail in, for example, Remington's
10 Pharmaceutical Science, 15th Ed., Mack Publishing Company, Easton, Pennsylvania (1990).

15 The antibodies of this invention can be lyophilised for storage and reconstituted in a suitable carrier prior to use. This technique has been shown to be effective with conventional immunoglobulins. Any suitable lyophilisation and reconstitution techniques can be employed. It will be appreciated by those skilled in the art that lyophilization and reconstitution can lead to varying degrees of antibody activity loss (eg. with conventional immune globulins, IgM antibodies tend to have greater activity loss than IgG antibodies) and that use
20 levels may have to be adjusted to compensate.

The dosage range of the antibody in accordance with the invention is about 0.5 to 1000 mg/m², preferably about 0.5 to 250mg/m², more preferably, between 0.5
25 and 100mg/m² and 0.5 and 50mg/m² and most preferably between 5 and 25mg/m² such as for example, 15mg/m².

Similarly, expressed in mg per dose, the dosages of the antibody may be about 1 to 2000 mg per dose, preferably about 1 to 500 mg per dose, more preferably
30 between 1 to 200 mg per dose and between 1 to 100mg per dose and most preferably between 10 and 50mg per dose such as, for example 30 mg per dose.

Single or multiple administrations of the compositions can be carried out with
35 dose levels and pattern being selected by the treating physician. In any event,

the pharmaceutical formulations should provide a quantity of the antibody(ies) sufficient to effectively treat the patient.

5 Typically, the chemotherapeutic agent and antibody will be presented as separate pharmaceutical compositions for co- administration, but they may also be formulated as a single pharmaceutical formulation. In this way both the antibody and the chemotherapeutic agent are presented to the patient within one and the same composition.

10 One or more distinct chemotherapeutic agents and one or more distinct anti-Ep-CAM antibodies may be co-administered in all aspects of the present invention. Thus reference to a chemotherapeutic agent comprises one or more distinct chemotherapeutic agent(s). If there is more than one chemotherapeutic agent, these may be administered either individually each on its own and/or together as
15 a chemotherapeutic agent cocktail. Similarly, reference to antibody comprises one or more distinct anti-Ep-CAM antibody(ies). If there is more than one antibody, these may again be administered either individually each on its own and/ or together as a cocktail. Additionally, the chemotherapeutic agent(s) are typically administered separately from the antibody(ies) but they may also be
20 administered together as a chemotherapeutic agent(s)/antibody(ies) cocktail.

Co-administration of the chemotherapeutic agent/radiotherapy with the antibody comprehends administration substantially simultaneously of both the chemotherapeutic agent/radiotherapy and the antibody. Essentially, the rational
25 behind co-administration is to allow sufficient exposure of Ep-CAM expressing tumour cells to a chemotherapeutic agent/radiotherapy known to block cell cycle progression at G₂ /M to achieve the desired increase in Ep-CAM antigen density prior to exposure of the same tumour cells to an anti-Ep-CAM antibody thereby achieving greater targeting of anti-Ep-CAM antibodies to Ep-CAM expressing
30 tumours. Co-administration therefore comprises any mode of administering a chemotherapeutic agent/radiotherapy in conjunction with an anti-Ep-CAM antibody that will achieve this result. Throughout the specification the term "combination of an anti-Ep-CAM antibody with a chemotherapeutic agent" refers to one wherein the chemotherapeutic agent/radiotherapy and the anti-Ep-CAM
35 antibody have been co-administered.

5 Preferably the chemotherapeutic agent is administered simultaneously with the antibody or more preferably before the antibody. Thus the chemotherapeutic agent may be administered on the same day as the antibody, either together or within hours of each other but may also be administered up to about two months beforehand, typically, about one or two weeks beforehand and more typically less than a week beforehand, say one to three days beforehand.

10 Additionally, co-administration also includes administering more than one dose of antibody within several weeks after one or more doses of chemotherapeutic agent, in other words the chemotherapeutic agent need not be re-administered again with every subsequent administration of the antibody, but may be administered just once or intermittently during the course of antibody treatment. Co-administration also comprises administration of the chemotherapeutic agent
15 up to 3 weeks after the antibody, preferably within a week and more preferably within a few days such as one to five days.

20 The antibody may be administered several times daily. Similarly the chemotherapeutic agent may be infused continuously over several hours or even days.

25 The present invention also provides a method of treating mammalian patients, preferably humans, afflicted with cancer which comprises co-administering a chemotherapeutic agent which is capable of arresting Ep-CAM antigen expressing cells in G₂/M in combination with an anti-Ep-CAM antibody. Preferably, the chemotherapeutic agent is given simultaneously and more preferably prior to administration of the antibody.

30 The cancers which may be treated particularly effectively with this combination therapy are primary or metastatic cancers of any histologic or histogenetic origin that express the Ep-CAM antigen. This includes, for example, prostate cancers, lung cancers, breast cancers, colon cancers, pancreatic cancers and ovarian cancers.

Dosing schedules for the treatment method of the present invention can be adjusted to account for the patient characteristics, disease state, characteristics of the chemotherapeutic agent and characteristics of the anti-Ep-CAM antibody. The goal of dosing schedules under this invention will be to administer anti-Ep-CAM antibody in a manner that will expose the Ep-CAM expressing tumour cells to the anti-Ep-CAM antibody at a time when antigen expression is likely to be increased due to exposure to chemotherapy which is known to block cell cycle progression at G₂/M. Additionally, as much as possible a dosing schedule convenient for the patient must be maintained.

Preferred dosing schedules for administration of the anti-Ep-CAM antibody and chemotherapy include: administering the anti-Ep-CAM antibody once every one or two weeks, preferably once every three or four weeks or a combination thereof for as long as necessary. The chemotherapeutic agent is given according to the established regimen for that agent or a regimen which will allow exposure of Ep-CAM expressing tumour cells to be arrested in G₂/M. Preferred dosing schedules vary with the chemotherapy agent and disease state but include, for example, once weekly, once every three or four weeks, or daily for several (e.g. 3-5) days repeated every three or four weeks for as long as necessary. Dosing of the anti-Ep-CAM antibody may take place on the same day or different days as indicated for the chemotherapeutic agent. Adjustment of the dosing schedule or strength of dose to prevent or decrease toxicity or side effects may take place with either the anti-Ep-CAM antibody or the chemotherapy agent.

For example, the preferred dosing schedule for co-administration of vinorelbine and cisplatin in combination with humanised 323/A3 (IgG₁) is administration of humanised 323/A3 (IgG₁) at a dose of 30mg/m² once a week for as long as necessary but typically for a period of 3 to 4 weeks, followed by a 30mg/m² dose every other week thereafter for as long as necessary. Vinorelbine is administered at a dose 25mg/m² on day 1, 8, 15 and 22. Cisplatin is given only once at a dose of 100mg/m² on day 1. Thereafter the vinorelbine /cisplatin regime is repeated every 28 days for as long as necessary. Preferably, vinorelbine, cisplatin and humanised 323/A3 (IgG₁) are administered at the same time on day one over a period of about 2 to 3 hours.

Another example of a preferred dosing schedule is the administration of paclitaxel/carboplatin in combination with humanised 323/A3 (IgG₁), wherein 323/A3 (IgG₁) is administered as for the vinorelbine/cisplatin example above and paclitaxel and carboplatin are given at a dose of 225 mg/m² and AUC = 6.0 respectively, on day 1, with a repeat dosage every 28 days thereafter for as long as necessary. Again, paclitaxel, carboplatin and humanised 323/A3 (IgG₁) are preferably administered together on day 1 over a period of about 2 to 3 hours.

Other preferred dosage schedules which comprise the combination of 323/A3 (IgG₁) with any of navelbine, cisplatin or taxol on their own would comprise similar dosages and administration schedules, using just one anticancer agent instead of two.

When the preferred anti-Ep-CAM antibody is Panorex, the dosage of antibody is between 10 to 500mg per dose, preferably 100mg per dose.

A further aspect of the present invention is a method of increasing antibody binding of anti-Ep-CAM antibodies to Ep-CAM expressing cells by co-administering to a patient a chemotherapeutic agent capable of arresting cells in G₂/M together with said anti-Ep-CAM antibody.

By co-administering a chemotherapeutic agent according to the present invention together with an Ep-CAM antibody, it is possible to increase antibody binding by about 2 to 10 fold, preferably by more than 4 fold, more preferably by more than 6 fold and most preferably by more than 8 fold.

Figures

Figure 1.

Ep-CAM is expressed across the cell cycle, but at higher density and greater homogeneity on cells in S (dotted line) and in G₂/M (dashed line) phases than in G₀/G₁ cells (solid line). This pattern of expression has been documented in a number of other human colon, prostate, and lung tumour cell lines.

Figure 2.

Cell cycle arrest is a prominent feature of adenocarcinoma cells exposed in vitro to Navelbine (NVB; 30 nM) plus Cisplatin (CDDP; 5 μ M), or Taxol (TAX; 80 nM) plus Carboplatin (CPBDA; 100 μ M), compared to media alone, 5-Fluorouracil (5FU), interferon-alpha (IFN-alpha; 100 U/ml), or interferon-gamma (IFN-gamma; 100 U/ml). The area of each bar is divided to indicate the percentage of cells in G₀/G₁ and in S + G₂/M phases; the height of each bar indicates the average number of Ep-CAM molecules per cell within the population. Cells in S phase and in G₂/M phase express higher levels of Ep-CAM (Figure 1), and the agents which blocked cell cycle progression had overall increased Ep-CAM expression

Figure 3.

The expression of Ep-CAM antigen was quantified on a variety of adenocarcinoma cell lines as well as primary cultures of normal human cells. Cultured cells were exposed sequentially to media, or to 30 nM Navelbine followed by 5 μ M Cisplatin (NVB + CDDP), or to 80 nM Taxol followed by 100 μ M Carboplatin (TAX + CPBDA). The 4 adenocarcinoma cells expressed higher antigen levels subsequent to exposure to cycle-specific drug combinations, whereas the 4 normal cells did not show any increase in antigen expression, which remained undetectable in 2 of the normal cell populations.

Figure 3a.

The binding of Panorex, a related murine monoclonal antibody with specificity for the Ep-CAM antigen, was evaluated after a 15 minute incubation with HT29 adenocarcinoma cells which had been cultured with Navelbine plus Cisplatin or with Taxol as previously described. A significant increase (34%) in antibody binding was seen on the cells treated with Navelbine plus Cisplatin; 82% of these cells were arrested in S or G₂/M cycle phase compared to 21% of the control cells. (A smaller increase (8%) in antibody binding was seen for cells treated with Taxol, but in this experiment only 57% of the cells were cycle-arrested) as is shown in Figure 3a.

Figure 4.

The ability of human peripheral blood ADCC effector cells to lyse tumour target cells incubated with humanized 323/A3 (IgG₁) (a humanized monoclonal antibody having specificity for the Ep-CAM antigen and capable of interacting with Fc receptors on human effector cells) in vitro was improved when the target cells had been pre-treated with NAVELBINE (30 nM) plus Cisplatin (5 µM).

Figure 5.

Treatment of human tumour xenograft-bearing mice with a cell-cycle-specific cytotoxic agent promoted improved localization of antibody specific for Ep-CAM to the tumours.

Figure 6.

Humanised 323/A3 (IgG₁) Kappa Light Chain Amino Acid Sequence

Figure 7.

Humanised 323/A3 (IgG₁) Heavy Chain Amino Acid Sequence

Figure 8.

Vector Map of pEE6

Figure 9.

Vector Map of pEE12

Figure 10.

Vector Map of pEE18

Figure 11

Humanised 323/A3 (IgG_{4cys}) Kappa Light Chain Amino Acid Sequence

Figure 12

Humanised 323/A3 (IgG_{4cys}) variant Heavy Chain Amino Acid Sequence

Figure 13

Humanised 323/A3 (IgG_{2cys}) Kappa Light Chain Amino Acid Sequence

Figure 14

Humanised 323/A3 (IgG_{2cys}) Heavy Chain Amino Acid Sequence

5 Figure 15

Humanised 323/A3 (IgG₁) light chain cDNA Sequence

Figure 16

Humanised 323/A3 (IgG₁) Heavy chain cDNA Sequence

10

Figure 17

Humanised 323/A3 (IgG₄) heavy chain cDNA Sequence

Figure 18

15 Humanised 323/A3 (IgG_{2cys}) heavy chain cDNA Sequence

The following examples illustrate the invention.

20 **Example 1. Ep-CAM antigen expression varied by phase across the cell cycle on PC-3 prostatic adenocarcinoma cells.**

Populations of PC-3 prostatic adenocarcinoma cells were evaluated for distribution in G₀/G₁, S, and G₂/M phases of the cell cycle as well as Ep-CAM expression. Cells were gently trypsinized and mechanically detached from the culture flasks and resuspended in calcium-and magnesium-free phosphate-buffered saline containing bovine serum albumin and NaN₃. Exactly 2 x 10⁵ cells were stained with FITC-323/A3 murine IgG antibody or FITC-murine IgG (control). Cells were fixed with cold paraformaldehyde, then permeabilized for DNA staining with Tween-20. Cellular DNA was stained with propidium iodide and RNase A. Listmode data were acquired on a FACScan flow cytometer (Becton Dickinson Immunocytometry Systems) equipped with a 488 nm laser using Cell Fit software. Cell cycle analysis was done using SOBR modelling (where possible, otherwise manual estimations were employed) on Cell Fit.

Ep-CAM antigen expression as detected by 323/A3 binding was evaluated separately using histogram analysis in Win List (Verity Software House).

Figure 1 shows that Ep-CAM is expressed across the cell cycle, but at higher density and greater homogeneity on cells in S (dotted line) and in G₂/M (dashed line) phases than in G₀/G₁ cells (solid line). This pattern of expression has been documented in a number of other human colon, prostate, and lung tumor cell lines.

Example 2. Increased expression of Ep-CAM antigen on adenocarcinoma cells was associated with arrest of cell cycle progression and accumulation of cells in S and G₂/M phases.

Adenocarcinoma cell lines were exposed to the various drugs or combinations of drugs as indicated in Figure 2. Subconfluent cells were exposed to Navelbine or Taxol for up to 24 hours, then washed and exposed to Cisplatin or Carboplatin, respectively, overnight. Cells were exposed to 5FU for 24 hours, and for 2-5 days to the interferons. Cells were washed and cultured for another 2-5 days prior to analysis for antigen expression and cell cycle status as described in Example 1. Antigen expression was quantified by comparison of the binding of fluorescein-conjugated 323/A3 to cultured cells with binding to calibrated microbead standards.

Cell cycle analysis demonstrated that only 6.3% of the media control cells were in S and G₂/M phases combined, compared to 39.4% of NVB + CDDP and 82.6% of TAX + CPBDA cells, both combinations of which caused significant increases in Ep-CAM antigen expression (as demonstrated in Figure 2). Antigen expression was not significantly increased in cells exposed to 5FU, IFN- α , or IFN- γ , which had only 7.9%, 12%, and 11.5%, respectively, of cells in S + G₂/M phase. Thus, only the drugs which caused accumulation of cells in S or G₂/M phases were able to cause a significant increase in Ep-CAM antigen expression.

Example 2a.

The binding of Panorex, a related murine monoclonal antibody with specificity for the Ep-CAM antigen, was evaluated after a 15 minute incubation with HT29
5 adenocarcinoma cells which had been cultured with Navelbine plus Cisplatin or
with Taxol as previously described. A significant increase (34%) in antibody
binding was seen on the cells treated with Navelbine plus Cisplatin; 82% of
these cells were arrested in S or G₂/M cycle phase compared to 21% of the
control cells. (A smaller increase (8%) in antibody binding was seen for cells
10 treated with Taxol, but in this experiment only 57% of the cells were cycle-
arrested) as is shown in Figure 3a.

**Example 3. Increased Ep-CAM antigen expression was observed on
tumour cells but not normal cells exposed to cytotoxic
15 drugs in vitro.**

The expression of Ep-CAM antigen was quantified on a variety of
adenocarcinoma cell lines as well as primary cultures of normal human cells.
Cultured subconfluent cells were exposed sequentially to media, or to 30 nM
20 Navelbine followed by 5 µM Cisplatin (NVB + CDDP), or to 80 nM Taxol followed
by 100 µM Carboplatin (TAX + CPBDA). Cells were washed with media and
cultured for another 2-5 days prior to analysis for antigen expression as
described in Examples 1 and 2.

25 Figure 3 clearly shows that the 4 adenocarcinoma cells expressed higher
antigen levels subsequent to exposure to cycle-specific drug combinations,
whereas the 4 normal cells did not show any increase in antigen expression,
which remained undetectable in 2 of the normal cell populations

**30 Example 4. Cells exposed to NAVELBINE plus Cisplatin were better
targets for human ADCC activity than control cells.**

Adenocarcinoma cells were exposed to drugs as described in Examples 1 and
2 above, and then harvested and seeded into 96-well plates for use as target
35 cells in a ⁵¹Cr-release cytotoxicity assay. Target cells were cultured overnight

with ^{51}Cr , and then washed. Human peripheral blood mononuclear cells which had been allowed to adhere overnight were added at a 50:1 effector: target ratio, and the ADCC cultures were incubated for 6 hours. Supernatants were collected and counted for radioactivity, and the percentage specific release was calculated. (see Figure 4).

Figure 4 clearly shows that PC-3 prostatic adenocarcinoma cells are better targets for human ADCC activity after exposure to Navelbine/Cisplatin compared to controls which have not been exposed to these chemotherapeutic agents. This effect may be due directly to increased antigen expression and thereby increased antibody binding, decreased modulation of the Ep-CAM antigen, increased fragility of the target cells, or a combination of the above.

Example 5. Antibody targeting to Ep-CAM-positive tumours was significantly improved by pre-treatment of the mice with NAVELBINE.

Human colon adenocarcinoma (HT-29) tumours were initiated by subcutaneous implantation into female CD-1 nude mice (Charles River). When the tumours reached 200-300 mg, animals were divided into groups of five. Navelbine was injected intravenously at a dosage of 28 mg/kg on days 1 and 5. A control group was dosed with 5-fluorouracil (5-FU) intraperitoneally at 20 mg/kg on days 1 and 5. On day 6, humanised 323/A3 IgG_{4Cys-TMT} (a humanized monoclonal antibody chelator conjugate with specificity for the Ep-CAM antigen) was labelled with lutetium-177 and injected intravenously via the lateral tail vein. Each mouse received 4.1 μg protein/2.09 μCi lutetium-177/0.2 ml injection. Blood, spleen, liver, lung, kidney, femur and tumour were harvested on days 1, 3 and 5 post-antibody for direct gamma counting (see Figure 5 for results).

Figure 5 shows that pre-treatment with Navelbine increases antibody targeting to Ep-CAM positive tumours whilst pre-treatment with 5-FU does not.

Example 6. Expression of the Humanized Antibody 323/A3 (IgG₁) variant in NSO Cells

1. Purpose/Summary

The cDNAs encoding the humanized 323/A3 antibody light and heavy chains (see Figures 15 and 16 respectively) were genetically engineered into a single Celltech glutamine synthetase (GS) expression plasmid, pEE18 (see Fig. 10), and used to transfect murine NSO cells.

2. Materials and Methods

2.1 Materials

NSO cells were obtained from Celltech Biologics plc, Slough, SL1 4EN, Berkshire, UK. The expression plasmids pEE6HCMV and pEE12 (see Figures 8 and 9) were obtained from Celltech Biologics plc, Slough.

2.2 The pEE6hmcv plasmid (see Figure 8) encoding full length humanised heavy chain DNA was digested with *Bam* *HI* and *Bgl* *II* to liberate the 3.2 kb fragment that contained the DNA encoding the heavy chain under the transcriptional control of the major immediate early promoter of the human cytomegalovirus. This fragment was cloned into the *Bam* *HI* site of pEE12 (Figure 9) that contained the DNA encoding the humanised light chain. (See Figure 6 for humanised 323/A3 (IgG₁) Kappa light chain amino acid sequence and Figure 7 for the humanised 323/A3(IgG₁) Heavy chain amino acid sequence. See Figure 10 for schematic representation of the pEE18 plasmid encoding 323/A3 (IgG₁) heavy and light chains.

2.2.2 Transfection and Selection of NSO Cells

2.2.2.1 Tissue Culture

All single cell culture activities were performed in isolated rooms that contained a single laminar flow hood and single incubator dedicated solely to the use of NSO cells in the production of stable cell lines secreting humanised 323/A3(IgG₁). No other NSO cells lines, human

cell lines or virus transformed cell lines were used within this environment.

5 A vial of NSO cells was revived and grown in 1:1:1 medium composed of DMEM:RPMI-1640:Sigma PFHM (1:1:1) to a cell density between 0.5 and 1×10^6 mL. For electroporation, the cells were harvested by centrifugation and washed once with PBS. pEE18 plasmid DNA encoding 323/A3 (IgG₁) was digested with Sal I, heat inactivated at 65°C for 15 minutes, precipitated with ethanol and air-dried. The dried DNA pellet was resuspended in PBS to a concentration of 0.5 µg/mL and 100 µL aliquoted into a 2mm electroporation cuvette (BTX). Washed NSO cells were resuspended at 1.2×10^7 /ml and 400 µL added to the cuvette to give a final density of 10^6 mL in a final volume of 0.5 mL. Electroporation was at 300 V for 1 msec in a BTX 8209 GenePulser followed by incubation on ice for 5-10 minutes. The electroporation mixture was resuspended at 10^5 cells /mL with 1:1:1 medium and distributed over 96-well plates at 50 µL/well. The following day, wells were fed with 150 µL GS medium (Gln-free IMDM, 1= X GS and nucleoside supplement, 5% DFBS) to begin the GS selection process such that all wells had a final concentration of 3% DFBS.

2.2.2.2 Specific Production Rate (SPR)

25 Selected cell lines grown in GS media (3% DFBS) were seeded at a density of 0.2×10^6 cells/mL in T-25 flasks (Costar) that contained 5 mL of GS media (3% DFBS). Cells were incubated overnight at 37°C for 24 hours after which an aliquot of each culture supernatant was removed. The supernatants were used in the human IgG ELISA assay to determine the concentration of secreted humanised 323/A3(IgG₁). The SPR value was derived by multiplying the concentration of 323/A3 (IgG₁) antibody in the supernatant times the volume (5.0) and is expressed as µg/ 10^6 cells/24 hours.

2.2.2.3 Cryopreservation of Cells

5 Selected cell lines were routinely harvested when cell density was greater than 0.2×10^6 cells/mL. An appropriate volume of cells was removed and subjected to centrifugation at $1,000 \times g$ for 5 minutes at 22°C . The cell pellet was gently resuspended to $1 - 4 \times 10^6$ cells/mL with ice-cold freezing media consisting of 20% (v/v) FBS/ 10-% (v/v) DMSO/ GS Media (sterile filtered). Each 1.0mL of the cell suspension was aliquoted into a 1.8 ml cryopreservation vial (NUNC) and gradually frozen overnight in a Cryo 1°C Freezing Container (Nalgene) that had been placed in a -70°C freezer. The vials were then removed from the container and stored in the vapour phase of a liquid nitrogen freezer.

15 Twenty vials of each cell line, including a low humanised 323/A3(IgG₁) producer were frozen down as described above and stored initially in the vapour phase of an MVE Cryogenics XLC440 liquid nitrogen freezer. The cells were subsequently transferred and stored in the vapour phase of an MVE Cryogenics XLC500 liquid nitrogen freezer.

20 Example 7. Expression of the Humanized Antibody 323/A3(IgG_{4cys}) in NSO Cells

1. Purpose Summary

25 The cDNAs encoding the humanized antibody 323/A3(IgG_{4cys}) (a humanised 323/A3 antibody) antibody light and heavy chains (see Figures 15 and 17) were genetically engineered into a single Celltech glutamine synthetase (GS) expression plasmid, pEE18, and used to transfect murine NSO cells.

2. Materials and Methods

2.1 Materials (as for Example 6 above)

2.2 Creation of humanised 323/A3 (IgG_{4cys}) pEE18 Expression Plasmid

30 The pEE6HMCV plasmid (see Figure 8) encoding full length humanized heavy chain DNA was digested with *BAM* *HI* and *Bgl* *II* to liberate a 3.2 kb fragment that contained the DNA encoding the heavy chain under the transcriptional control of the major immediate early

35

promoter of the human cytomegalovirus. This fragment was cloned into the *Bam HI* site of pEE12 that contained the DNA encoding the humanized light chain (See Figure 11 for humanised 323/A3(IgG₄) Kappa Light Chain Amino Acid Sequence and Figure 12 for the 323/A3 IgG_{4cys} variant Heavy Chain Amino Acid Sequence). See Figure 10 for schematic representation of the pEE18 plasmid encoding 323/A3 heavy and light chains.

2.2.2 Transfection and Selection of NSO Cells: see Example 6 above.

Example 8. Expression of the Humanized Antibody 323/A3(IgG_{2cys}) in NSO Cells

1. Purpose/Summary

The cDNAs encoding the humanized 323/A3(IgG_{2cys}) antibody heavy and light chains were genetically engineered into a single Celltech glutamine synthetase (GS) expression plasmid, pEE18, and used to transfect murine NSO cells.

2. Materials and Methods

2.1 Materials as for Examples 6 and 7 above

2.2 Creation of 323/A3 (IgG_{2cys}) pEE18 Expression for Plasmid

The pEEE6 hcmv plasmid encoding full-length humanized heavy chain DNA was digested with *Bam HI* and *Bgl II* to liberate 3.2 kb fragment that contained the DNA encoding the heavy chain under the transcriptional control of the major immediate early promoter of the human cytomegalovirus. This fragment was cloned into the *Bam II* site of pEE12 that contained the DNA encoding the humanized light chain (See Figure 13 for 323/A3(IgG_{2cys}) Kappa Light Chain Amino Acid Sequence and Figure 14 for the 323/A3(IgG_{2cys}) Heavy Chain Amino Acid Sequence). See Figure 10 for schematic representation of the pEE18 plasmid encoding 323/A3 (IgG_{2cys}) heavy and light chains.

2.2.2 Transfection and Selection of NSO Cells - See Examples 6 and 7 above.

CLAIMS:

1. A combination of an anti-Ep-CAM antibody with a chemotherapeutic agent that is capable of arresting Ep-CAM antigen expressing cells in S or G₂/M.
5
2. A combination according to claim 1 wherein the Ep-CAM antibody is a 17.1A antibody.
3. A combination according to claim 2 wherein the Ep-CAM antibody is Panorex.
10
4. A combination according to any of the above claims wherein the chemotherapeutic agent is one or more agents selected from UFT, Capecitabine, CPT-II, Oxaliplatin, 5FU, 5FU continuous infusion, Paclitaxel, Docetaxel, Cyclophosphamide, Methotrexate, Doxorubicin, Navelbine (iv and oral), Epirubicin, Mitoxantrone, Raloxifen, Cisplatin, Mitomycin, Carboplatinum, Gemcitabine, Etoposide and Topotecan.
15
5. A combination according to claim 4, wherein the chemotherapeutic agent is CPT-II, 5FU (continuous infusion), Oxaliplatin, Capecitabine, UFT and Tomudex (Raloxifen).
20
6. A combination according to any of the above claims wherein the Ep-CAM expressing cells are cells of epithelial origin.
25
7. A combination according to any of the preceding claims wherein the Ep-CAM antigen expressing cells are tumour cells and their metastases.
8. A combination according to claim 7, wherein the Ep-CAM expressing tumour cells are adenocarcinoma cells and their metastases.
30
9. A combination according to claims 7 and 8, wherein the Ep-CAM expressing cells are prostate, lung, breast, gastric or colon originating cells or other tumours known to express the Ep-CAM antigen.
35

- 5 10. Use of an anti-Ep-CAM antibody in the manufacture of a medicament for use in anti-cancer therapy characterised in that a chemotherapeutic agent which is capable of arresting Ep-CAM antigen expressing cells in S or in G₂/M is co-administered to a patient with an anti-Ep-CAM antibody.
- 10 11. Use of an anti-Ep-CAM antibody according to claim 10 wherein the chemotherapeutic agent is administered prior to or simultaneously with the anti Ep-CAM antibody.
- 15 12. A method of increasing antibody binding of an anti-Ep-CAM antibody which comprises co-administering to a patient a chemotherapeutic agent capable of arresting cells in S or in G₂/M with an Ep-CAM antibody.
- 20 13. A method according to claim 11 which increases antibody binding between 2 to 10 fold compared to binding in the absence of said chemotherapeutic agent.
- 25 14. A method of treatment wherein a chemotherapeutic agent which is capable of arresting Ep-CAM antigen expressing cells in S or in G₂/M is co-administered to a patient with an anti-Ep-CAM antibody.
15. A pharmaceutical composition an anti-Ep-CAM antibody with a chemotherapeutic agent that is capable of arresting Ep-CAM antigen expressing cells in G₂/M.

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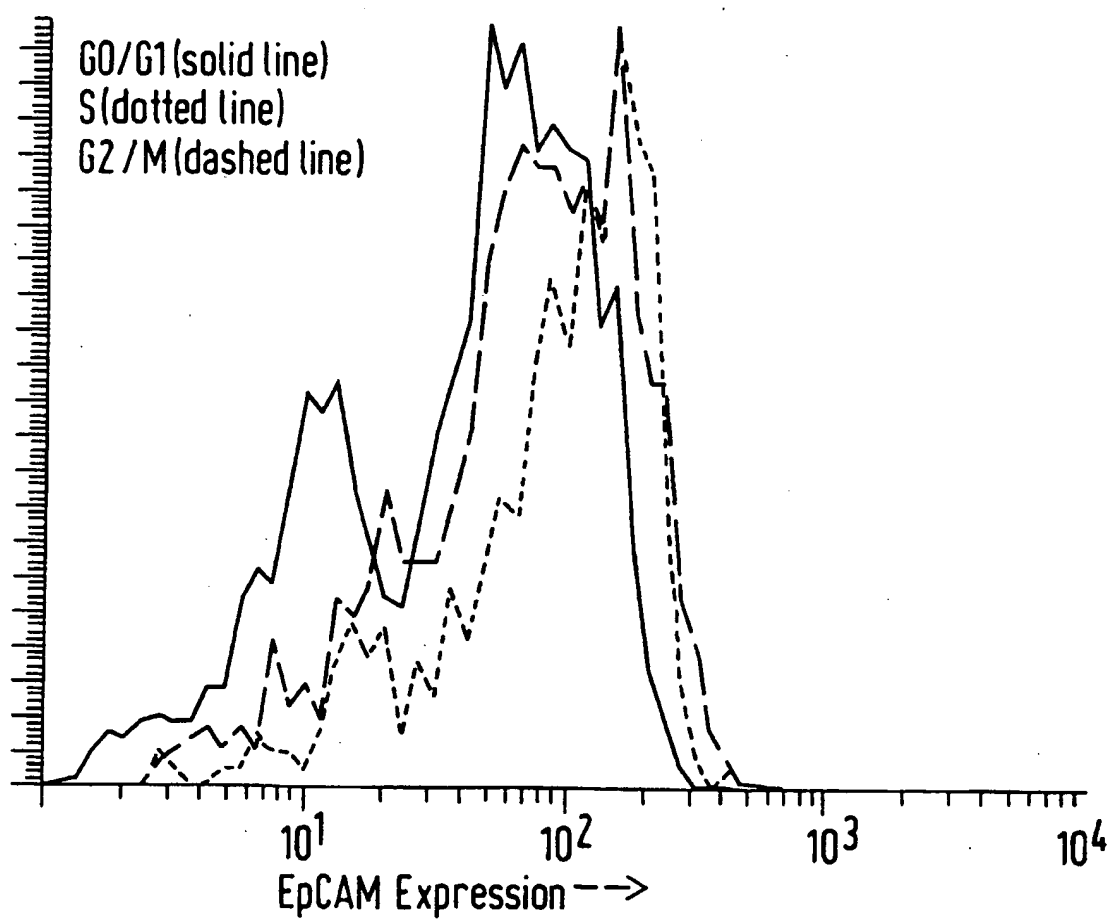


FIG. 1

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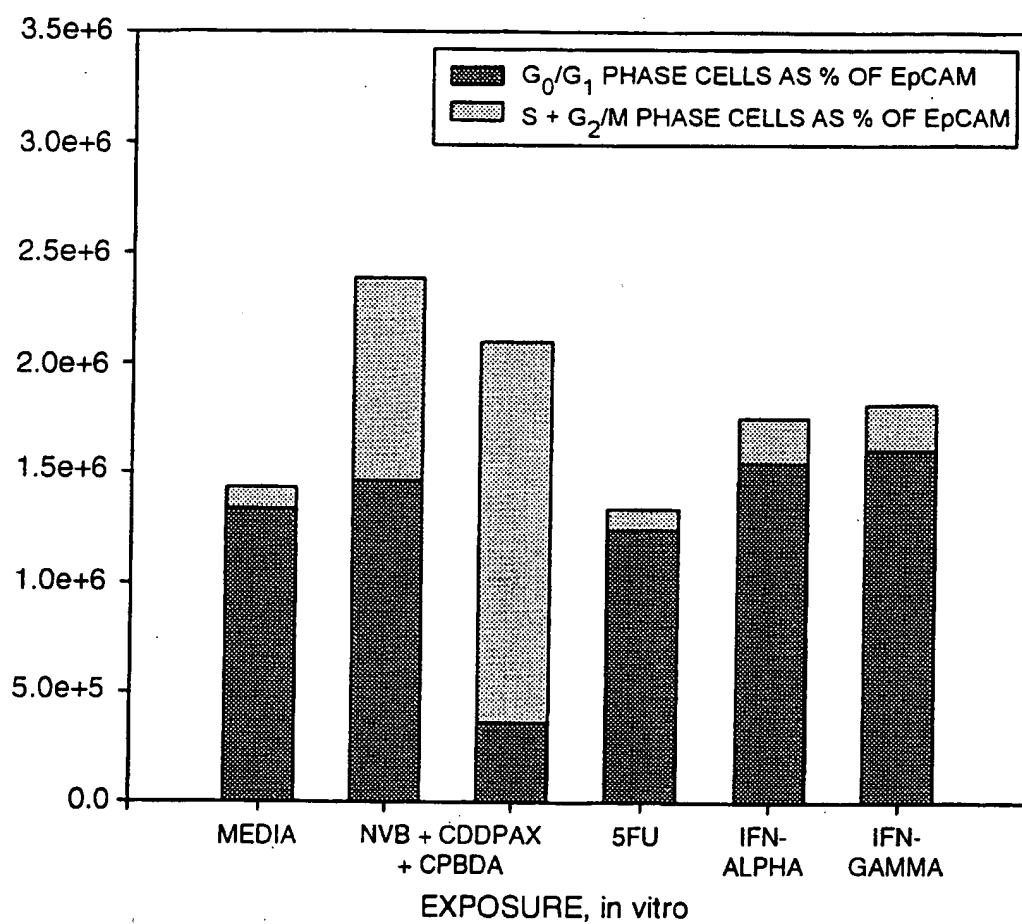


FIG. 2

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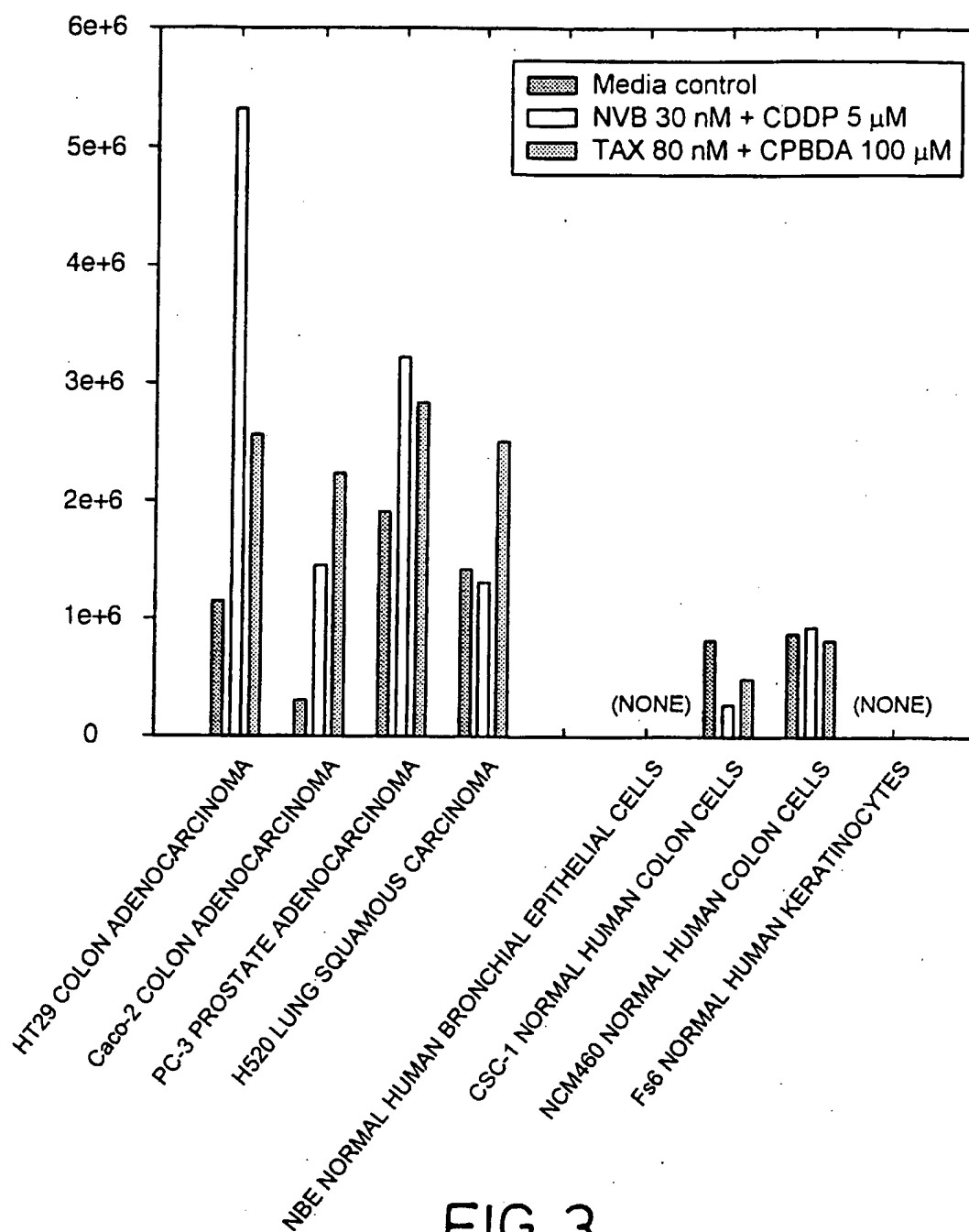


FIG. 3

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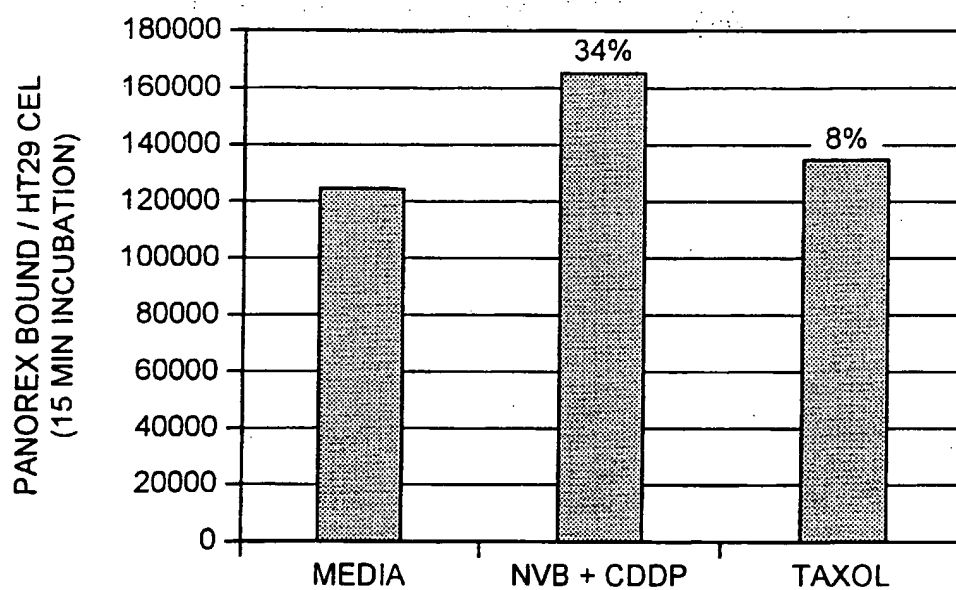


FIG. 3a

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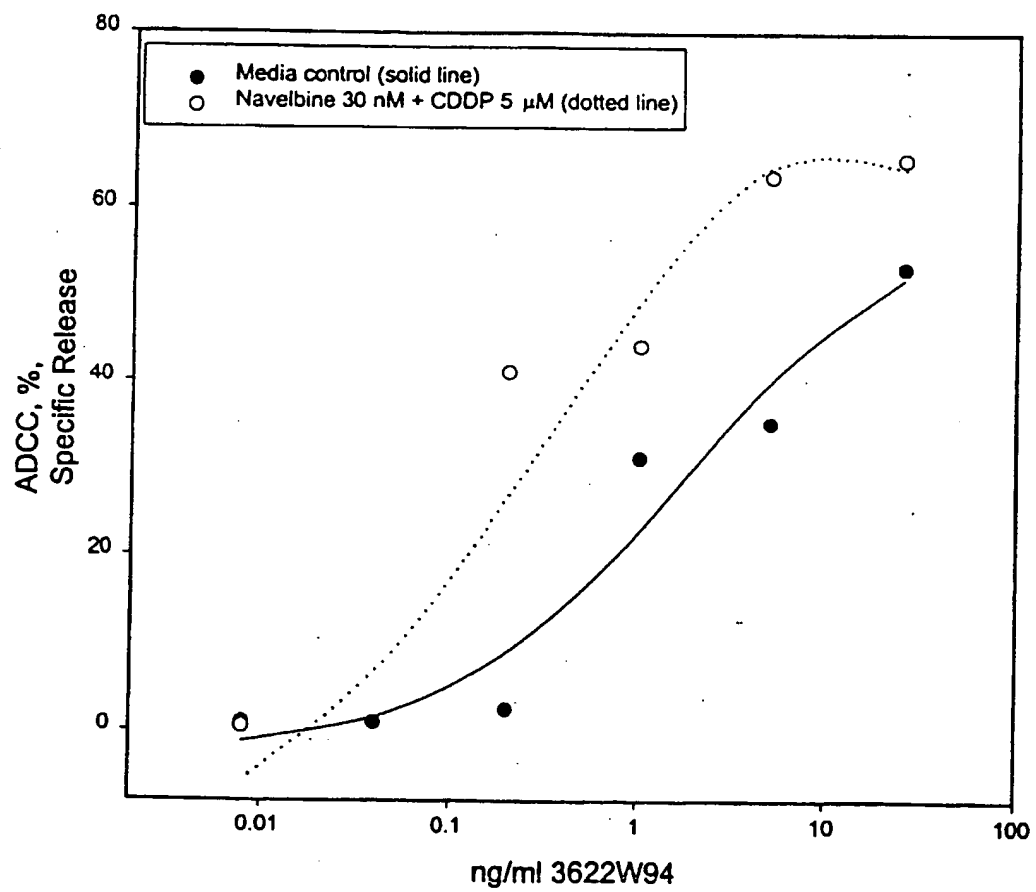


FIG. 4

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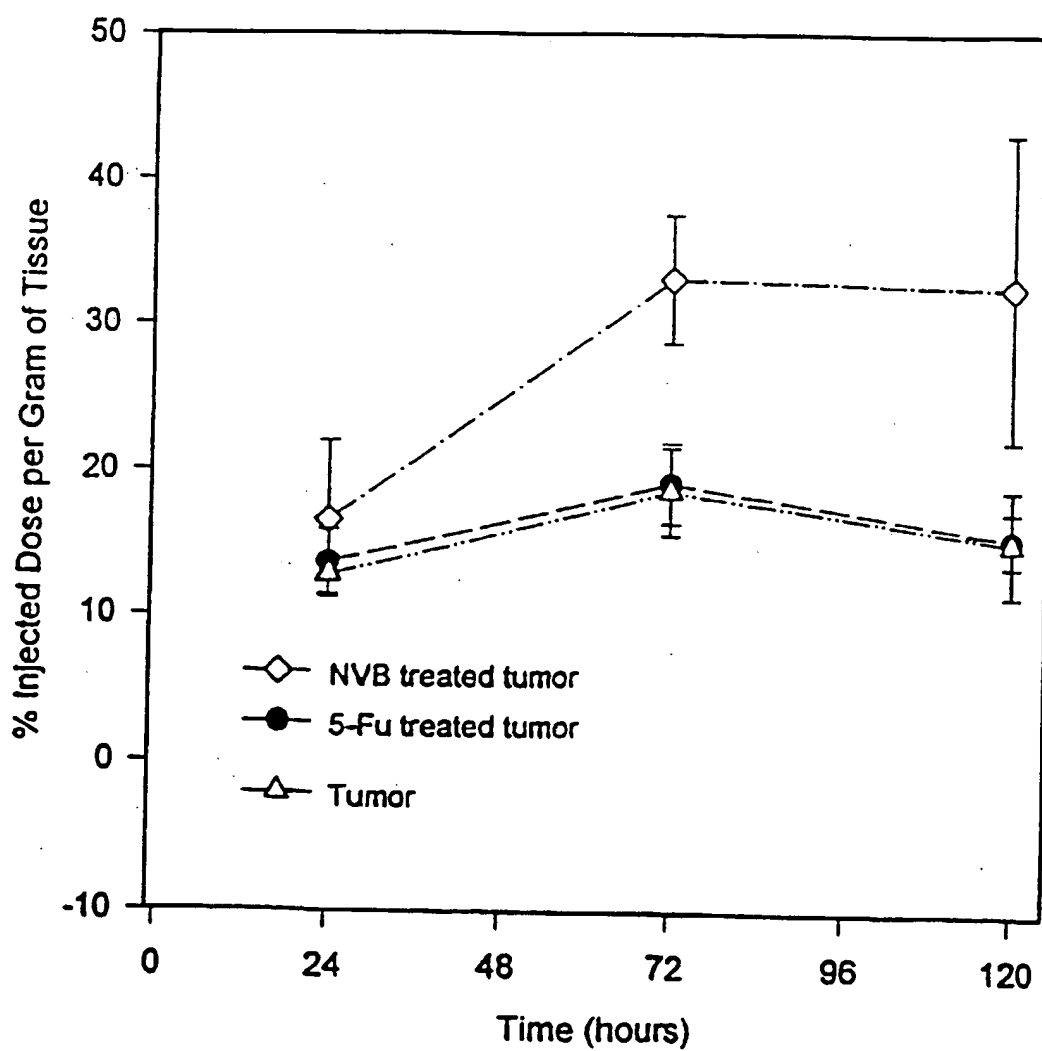


FIG.5

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Humanised 323/A3 (IgG₁) Kappa Light Chain Amino Acid Sequence

The amino acid sequence of the humanized light chain of 323/A3 IgG₁, including leader peptide, is shown below.

1	MGWSCILFL	VATATGVHSD	IVMTQSPLSL	PVTPGEPASI
41	SCRSSKNLLH	SNGITYLYWY	LQKPGQSPQL	LIYQMSNLAS
81	GVPDRFSSSG	SGTDFTLKIS	RVEAEDVGVY	YCAQNLEIPR
121	TFGQGGTKVEI	KRTVAAPSVF	IFPPSDEQLK	SGTASVVCLL
161	NNFYPPREAKV	QWKVDNALQS	GNSQESVTEQ	DSKDSTYSLS
201	STLTLSKADY	EKHKVVACEV	THQGLSSPVT	KSFNRGEC

FIG. 6

Humanised 323/A3 (IgG₁) Heavy Chain Amino Acid Sequence

The final amino acid sequence of the humanized heavy chain 323/A3 IgG₁, including leader peptide, is shown below.

1	MGWSCILFL	VATATGVHSQ	VQLVQSGPEV	KKPGASVKVS
41	CKASGYTFTN	YGMNWVRQAP	GQGLEWMGWI	NTYTGEPTYG
81	EDFKGRFAFS	LDSASTAYM	ELSSLRSED	AVYFCARFGN
121	YVDYWGQGS	VTVSSASTKG	PSVFPLASS	KSTSGGTAAL
161	GCLVKDYFPE	PVTVSWNSGA	LTSGVHTFPA	VLQSSGLYSL
201	SSVVTVPSSS	LGTQTYICNV	NHKPSNTKVD	KKVEPKSCDK
241	THTCPPCPAP	ELLGGPSVFL	FPPKPKDTLM	ISRTPEVTCV
281	VVDVSHEDPE	VKFNWYVDGV	EVHNAKTKPR	EEQYNSTYRV
321	VSVLTVLHQD	WLNGKEYKCK	VSNKALPAPI	EKTISKAKGQ
361	PREPQVYTL	PSRDELTKNQ	VSLTCLVKGF	YPSDIAVEWE
401	SNGQPENNYK	TTPPVLDSDG	SFFLYSKLTV	DKSRWQQGNV
441	FSCSVMHEAL	HNHYTQKSLS	LSPGK	

FIG. 7

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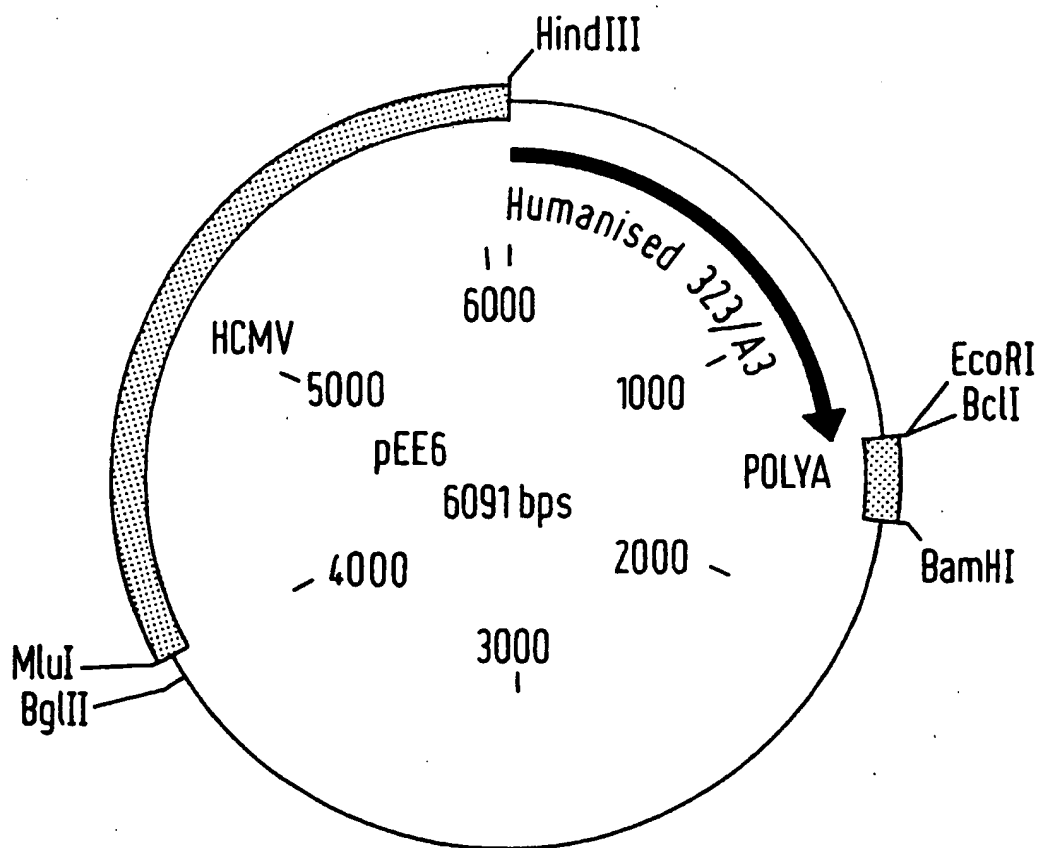


FIG. 8

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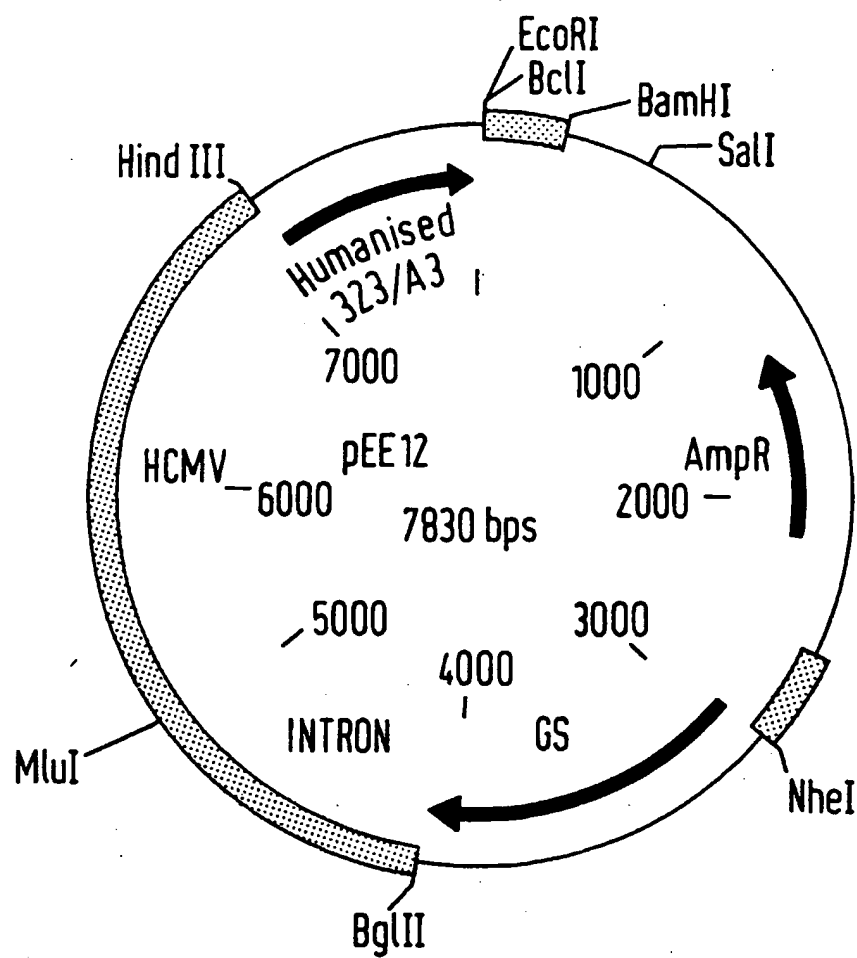


FIG. 9

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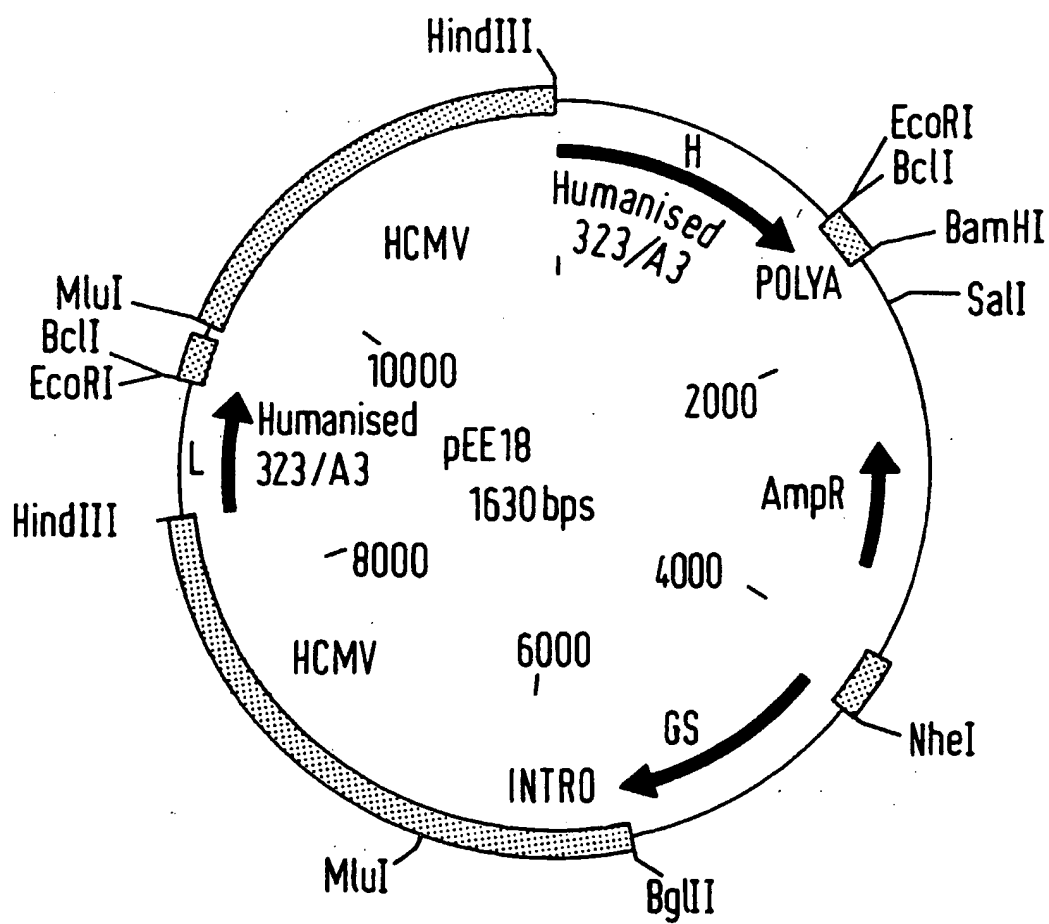


FIG. 10

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Humanised 323/A3 (IgG_{4cys}) Kappa Light Chain Amino Acid Sequence

The final amino acid sequence of the humanized light chain of 323/A3 IgG₄, including leader peptide, is shown below.

1	MGWSCILFL	VATATGVHSD	IVMTQSPLSL	PVTPGEPASI
41	SCRSSKNLLH	SNGITYLYWY	LQKPGQSPQL	LIYQMSNLAS
81	GVPDRFSSSG	SGTDFTLKIS	RVEAEDVGVY	YCAQNLEIPR
121	TFGQGTKVEI	KRTVAAPSVF	IFPPSDEQLK	SGTASVVCLL
161	NNFYPREAKV	QWKVDNALQS	GNSQESVTEQ	DSKDSTYSLS
201	STLTLSKADY	EKHKVYACEV	THQGLSSPVT	KSFNRGEC

FIG. 11

Humanised 323/A3 (IgG_{4cys}) Heavy Chain Amino Acid Sequence

The final amino acid sequence of the humanized heavy chain 323/A3 IgG₄, including leader peptide, is shown below.

1	MGWSCILFL	VATATGVHSQ	VQLVQSGPEV	KKPGASVKVS
41	CKASGYTFTN	YGMNWVRQAP	GQGLEWMGWI	NTYTGEPTYG
81	EDFKGRFAFS	LDTSASTAYM	ELSSLRSED	AVYFCARFGN
121	YVDYWGQGSL	VTVSSASTKG	PSVFPLAPCS	RSTSESTAAL
161	GCLVKDYFPE	PVTVSWNSGA	LTSGVHTFPA	VLQSSGLYSL
201	SSVTVPSST	LGTKTYTCNV	DHKPSNTKVD	KRVESKYGPP
241	CPPCPAPEFA	GAPSVFLFPP	KPKDTLMISR	TPEVTCVVVD
281	VSQEDPEVQF	NWYVDGVEVH	NAKTKPREEQ	FNSTYRVVSV
321	LTVLHQDWLN	GKAYKCKVSN	KGLPSSIEKT	ISKAKGQPRE
361	PQVYTLPPSQ	EEMTKNQVSL	TCLVKGFYPS	DIAVEWESNG
401	QPENNYKTP	PVLDSGDSFF	LYSRLTVDKS	RWQEGNVFSC
441	SVMHEALHNH	YTQKSLCLSL	GK	

FIG. 12

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Humanised 323/A3 (IgG_{2cys}) Kappa Light Chain Amino Acid Sequence

The final amino acid sequence of the humanized light chain of 323/A3 IgG_{2cys}, including leader peptide, is shown below.

1	MGWSCILFL	VATATGVHSD	IVMTQSPLSL	PVTPGEPASI
41	SCRSSKNLLH	SNGITYLYWY	LQKPGQSPQL	LIYQMSNLAS
81	GVPDRFSSSG	SGTDFTLKIS	RVEAEDVGVY	YCAQNLEIPR
121	TFGQGTKVEI	KRTVAAPSVF	IFPPSDEQLK	SGTASVVCLL
161	NNFYPPREKV	QWKVDNALQS	GNSQESVTEQ	DSKDSTYSLS
201	STLTLSKADY	EKHKVYACEV	THQGLSSPVT	KSFNRGEC

FIG. 13

Humanised 323/A3 (IgG_{2cys}) Heavy Chain Amino Acid Sequence

The final amino acid sequence of the humanized heavy chain of 323/A3 IgG_{2cys}, including leader peptide, is shown below.

1	MGWSCILFL	VATATGVHSQ	VQLVQSGPEV	KKPGASVKVS
41	CKASGYTFTN	YGMNWRQAP	GQGLEWMGWI	NTYTGEPTYG
81	EDFKGRFAFS	LDTASTAYM	ELSSLRSED	AVYFCARFGN
121	YVDYWGQGS	VTSSASTKG	PSVFPLAPCS	RSTSESTAAL
161	GCLVKDYFPE	PVTVSWNSGA	LTSGVHTFPA	VLQSSGLYSL
201	SSVTVPPSN	FGTQTYTCNV	DHKPSNTKVD	KTKVERKCCVE
241	CPPCPAPPVA	GPSVFLPPK	PKDTLMISRT	PEVTCVVVDV
281	SHEDPEVQFN	WYVDGVEVHN	AKTKPREEQF	NSTFRVVS
321	TVVHQDWLNG	KEYKCKVSNK	GLPAPAIEKTI	SKTKGQPREP
361	QVYTLPPSRE	EMTKNQVSLT	CLVKGFYPSD	IAVEWESNGQ
401	PENNYKTPPP	MLDSGGSFFL	YSKLTVDKSR	WQQGNVFCSS
441	VMHEALHNHY	TQKSLCLSLG	K	

FIG. 14

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Humanised 323/A3 (IgG₁) light chain DNA sequence
(also 323/A3 (IgG_{4cys} and IgG_{2cys} light chain cDNA sequence)

10	20	30	40	50
CGTAAGCTTC	ACAGGACCTC	ACC	ATG	GGA
GCATTCGAAG	TGTCCTGGAG	TGG	TAC	CCT
			Met	Gly
			Trp	
			Ser	
			Cys	
			Ile	
			Ile	
			Leu	
			Phe	
			AAC	
			Leu>	
60	70	80	90	100
GTA	GCA	ACA	GCT	ACA
CAT	CGT	TGT	CGA	TGT
Val	Ala	Thr	Ala	Thr
			Gly	
			Val	
			His	
			Ser>	
			Asp	
			Ile	
			Val	
			Met	
			Thr	
			Gln	
			Ser>	
110	120	130	140	
CCA	CTC	TCC	CTG	CCC
GGT	GAG	AGG	GAC	GGG
Pro	Leu	Ser	Leu	Pro
			Val	
			Thr	
			Pro	
			Gly	
			Glu	
			Pro	
			Ala	
			Ser	
			Ile	
			Ser	
			ACA	
			Cys>	
150	160	170	180	190
AGG	TCT	AGT	AAG	AAT
TCC	AGA	TCA	TTC	TTA
Arg	Ser	Ser	Lys	Asn
			Leu	
			Leu	
			His	
			Ser	
			Asn	
			Gly	
			Ile	
			Thr	
			Tyr	
			Leu	
			Tyr>	
200	210	220	230	240
TGG	TAC	CTG	CAG	AAG
ACC	ATG	GAC	GTC	TTC
Trp	Tyr	Leu	Gln	Lys
			Pro	
			Gly	
			Gln	
			Ser	
			Pro	
			Gln	
			Leu	
			Leu	
			Ile	
			Tyr	
			Gln>	
250	260	270	280	290
ATG	TCC	AAC	CTT	GCC
TAC	AGG	TTG	GAA	CGG
Met	Ser	Asn	Leu	Ala
			Ala	
			Ser	
			Gly	
			Val	
			Pro	
			Asp	
			Arg	
			Phe	
			Ser	
			Ser	
			Ser	
			Gly>	
300	310	320	330	340
TCA	GGC	ACA	GAT	TTT
AGT	CCG	TGT	CTA	AAA
Ser	Gly	Thr	Asp	Phe
			Thr	
			Leu	
			Lys	
			Ile	
			Ser	
			Arg	
			Val	
			Glu	
			Ala	
			Glu	
			Asp>	
350	360	370	380	
GTT	GGG	GTT	TAT	TAC
CAA	CCC	CAA	ATA	ATG
Val	Gly	Val	Tyr	Tyr
			Cys	
			Ala	
			Gln	
			Asn	
			Leu	
			Glu	
			Ile	
			Pro	
			Arg	
			Thr	
			Phe>	

FIG. 15

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390		400		410		420		430	
GGC	CAA	GGG	ACC	AAG	GTG	GAG	ATC	AAA	CGT
CCG	GTT	CCC	TGG	TTC	CAC	CTC	TAG	TTT	GCA
Gly	Gln	Gly	Thr	Lys	Val	Glu	Ile	Lys	Arg>
									Thr
									Val
									Ala
									Ala
									Pro
									Ser>
440		450		460		470		480	
GTC	TTC	ATC	TTC	CCG	CCA	TCT	GAT	GAG	CAG
CAG	AAG	TAG	AAG	GGC	GGT	AGA	CTA	CTC	GTC
Val	Phe	Ile	Phe	Pro	Pro	Ser	Asp	Glu	Gln
									Leu
									Lys
									Ser
									Gly
									Thr
									Ala>
490		500		510		520		530	
TCT	GTT	GTG	TGC	CTG	CTG	AAT	AAC	TTC	TAT
AGA	CAA	CAC	ACG	GAC	GAC	TTA	TTG	AAG	ATA
Ser	Val	Val	Cys	Leu	Leu	Asn	Asn	Phe	Tyr
									Pro
									Arg
									Glu
									Ala
									Lys
									GTA
									CAT
									Val>
540		550		560		570		580	
CAG	TGG	AAG	GTG	GAT	AAC	GCC	CTC	CAA	TCG
GTC	ACC	TTC	CAC	CTA	TTG	CGG	GAG	GTT	AGC
Gln	Trp	Lys	Val	Asp	Asn	Ala	Leu	Gln	Ser
									Gly
									Asn
									Ser
									Asn
									Ser
									Gln
									Glu
									Ser>
590		600		610		620			
GTC	ACA	GAG	CAG	GAC	AGC	AAG	GAC	AGC	ACC
CAG	TGT	CTC	GTC	CTG	TCG	TTC	CTG	TCG	TGG
Val	Thr	Glu	Gln	Asp	Ser	Lys	Asp	Ser	Thr>
630		640		650		660		670	
CTG	ACG	CTG	AGC	AAA	GCA	GAC	TAC	GAG	AAA
GAC	TGC	GAC	TCG	TTT	CGT	CTG	ATG	CTC	TTT
Leu	Thr	Leu	Ser	Lys	Ala	Asp	Tyr	Glu	Lys
									His
									Lys
									Val
									Tyr
									Ala
									Cys>
680		690		700		710		720	
GAA	GTC	ACC	CAT	CAG	GGC	CTG	AGC	TCG	CCC
CTT	CAG	TGG	GTA	GTC	CCG	GAC	TCG	AGC	GGG
Glu	Val	Thr	His	Gln	Gly	Leu	Ser	Ser	Pro
									Val
									Thr
									Lys
									Ser
									Phe
									Asn>
730		740							
AGG	GGA	GAG	TGT	TAG					
TCC	CCT	CTC	ACA	ATC					
Arg	Gly	Glu	Cys	>					

FIG. 15 cont.

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FIG. 16

Humanised 323/A3 (IgG₁) heavy chain DNA sequence

10	20	30	40	50	
CGTAAGCTTC	ACAGATCCTC	ACC	ATG Met	GGA Gly	TGG Trp
			AGC Ser	TGT Cys	ATC Ile
				ATC Ile	CTC Leu
				TTT Phe	CTG Leu>
60	70	80	90	100	
GTG Val	GCA Ala	ACA Thr	GCT Ala	ACA Thr	GGT Gly
			GTC Val	CAC His	TCC Ser>
				CAG Gln	GTA Val
				CAG Gln	CTA Leu
				GTG Val	CAA Gln
					TCA Ser>
110	120	130	140		
GGG Gly	CCT Pro	GAA Glu	GTG Val	AAG Lys	AAG Lys
				CCT Pro	GGG Gly
				GCC Ala	TCA Ser
				GTG Val	AAA Lys
				GTT Val	TCC Ser
					TGC Cys
					AAG Lys>
150	160	170	180	190	
GCT Ala	TCT Ser	GGC Gly	TAC Tyr	ACC Thr	TTC Phe
				ACC Thr	AAC Asn
				TAT Tyr	GGA Gly
				ATG Met	AAC Asn
				TGG Trp	GTA Val
				AGG Arg	CAG Gln>
200	210	220	230	240	
GCG Ala	CCT Pro	GGA Gly	CAG Gln	GGG Gly	CTT Leu
				GAG Glu	TGG Trp
				ATG Met	GGG Gly
				TGG Trp	ATA Ile
				AAC Asn	ACC Thr
					TAC Tyr
					ACT Thr>
250	260	270	280	290	
GGA Gly	GAG Glu	CCA Pro	ACA Thr	TAT Tyr	GGT Gly
				GAA Glu	GAT Asp
				TTC Phe	AAG Lys
				GGA Gly	CGG Arg
				TTT Phe	GCA Ala
					TTC Phe
					TCT Ser>
300	310	320	330	340	
CTA Leu	GAC Asp	ACA Thr	TCC Ser	GCC Ala	AGC Ser
				ACA Thr	GCC Ala
				TAT Tyr	ATG Met
				GAG Glu	CTC Leu
				AGC Ser	TCG Ser
				CTG Leu	AGA Arg>
350	360	370	380		
TCC Ser	GAG Glu	GAC Asp	ACT Thr	GCA Ala	GTC Val
				TAT Tyr	TTC Phe
				TGT Cys	GCG Ala
				AGA Arg	TTT Phe
				GGT Gly	AAC Asn
				TAC Tyr	GTA Val>
390	400	410	420	430	
GAC Asp	TAC Tyr	TGG Trp	GGT Gly	CAA Gln	GGA Gly
				TCA Ser	CTA Leu
				GTC Val	ACT Thr
				GTC Val	TCC Ser
				TCA Ser>	GCC Ala
					TCC Ser
					ACC Thr>

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440	AAG	GGC	CCA	TCG	GTC	TTC	CCC	CTG	GCA	CCC	TCC	TCC	AAG	AGC	ACC	TCT
	Lys	Gly	Pro	Ser	Val	Phe	Pro	Leu	Ala	Pro	Ser	Ser	Lys	Ser	Thr	Ser>
490	GGG	GGC	ACA	GCG	GCC	CTG	GGC	TGC	CTG	GTC	AAG	GAC	TAC	TTC	CCC	GAA
	Gly	Gly	Thr	Ala	Ala	Leu	Gly	Cys	Leu	Val	Lys	Asp	Tyr	Phe	Pro	Glu>
540	CCG	GTG	ACG	GTG	TCG	TGG	AAC	TCA	GGC	GCC	CTG	ACC	AGC	GGC	GTG	CAC
	Pro	Val	Thr	Val	Ser	Trp	Asn	Ser	Gly	Ala	Leu	Thr	Ser	Gly	Val	His>
590	ACC	TTC	CCG	GCT	GTC	CTA	CAG	TCC	TCA	GGA	CTC	TAC	TCC	CTC	AGC	AGC
	Thr	Phe	Pro	Ala	Val	Leu	Gln	Ser	Ser	Gly	Leu	Tyr	Ser	Leu	Ser	Ser>
630	GTG	GTG	ACC	GTG	CCC	TCC	AGC	AGC	TTG	GGC	ACC	CAG	ACC	TAC	ATC	TGC
	Val	Val	Thr	Val	Pro	Ser	Ser	Ser	Leu	Gly	Thr	Gln	Thr	Tyr	Ile	Cys>
680	AAC	GTG	AAT	CAC	AAG	CCC	AGC	AAC	ACC	AAG	GTG	GAC	AAG	AAA	GTT	GAG
	Asn	Val	Asn	His	Lys	Pro	Ser	Asn	Thr	Lys	Val	Asp	Lys	Lys	Val	Glu>
730	CCC	AAA	TCT	TGT	GAC	AAA	ACT	CAC	ACA	TGC	CCA	CCG	TGC	CCA	GCA	CCT
	Pro	Lys	Ser	Cys	Asp	Lys	Thr	His	Thr	Cys	Pro	Pro	Cys	Pro	Ala	Pro>
780	GAA	CTC	CTG	GGG	GGA	CCG	TCA	GTC	TTC	CTC	TTC	CCC	CCA	AAA	CCC	AAG
	Glu	Leu	Leu	Gly	Gly	Pro	Ser	Val	Phe	Leu	Phe	Pro	Pro	Lys	Pro	Lys>
830	GAC	ACC	CTC	ATG	ATC	TCC	CGG	ACC	CCT	GAG	GTC	ACA	TGC	GTG	GTG	GTG
	Asp	Thr	Leu	Met	Ile	Ser	Arg	Thr	Pro	Glu	Val	Thr	Cys	Val	Val	Val>
870	GAC	GTG	AGC	CAC	GAA	GAC	CCT	GAG	GTC	AAG	TTC	AAC	TGG	TAC	GTG	GAC
	Asp	Val	Ser	His	Glu	Asp	Pro	Glu	Val	Lys	Phe	Asn	Trp	Tyr	Val	Asp>

FIG. 16 cont.

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920	GGC	GTG	GAG	GTG	CAT	AAT	GCC	AAG	ACA	AAG	CCG	CGG	GAG	GAG	CAG	TAC
	Gly	Val	Glu	Val	His	Asn	Ala	Lys	Thr	Lys	Pro	Arg	Glu	Glu	Gln	Tyr>
970	AAC	AGC	ACG	TAC	CGT	GTG	GTC	AGC	GTC	CTC	ACC	GTC	CTG	CAC	CAG	GAC
	Asn	Ser	Thr	Tyr	Arg	Val	Val	Ser	Val	Leu	Thr	Val	Leu	His	Gln	Asp>
1020	TGG	CTG	AAT	GGC	AAG	GAG	TAC	AAG	TGC	AAG	GTC	TCC	AAC	AAA	GCC	CTC
	Trp	Leu	Asn	Gly	Lys	Glu	Tyr	Lys	Cys	Lys	Val	Ser	Asn	Lys	Ala	Leu>
1070	CCA	GCC	CCC	ATC	GAG	AAA	ACC	ATC	TCC	AAA	GCC	AAA	GGG	CAG	CCC	CGA
	Pro	Ala	Pro	Ile	Glu	Lys	Thr	Ile	Ser	Lys	Ala	Lys	Gly	Gln	Pro	Arg>
1110	GAA	CCA	CAG	GTG	TAC	ACC	CTG	CCC	CCA	TCC	CGG	GAT	GAG	CTG	ACC	AAG
	Glu	Pro	Gln	Val	Tyr	Thr	Leu	Pro	Pro	Ser	Arg	Asp	Glu	Leu	Thr	Lys>
1160	AAC	CAG	GTC	AGC	CTG	ACC	TGC	CTG	GTC	AAA	GGC	TTC	TAT	CCC	AGC	GAC
	Asn	Gln	Val	Ser	Leu	Thr	Cys	Leu	Val	Lys	Gly	Phe	Tyr	Pro	Ser	Asp>
1210	ATC	GCC	GTG	GAG	TGG	GAG	AGC	AAT	GGG	CAG	CCG	GAG	AAC	AAC	TAC	AAG
	Ile	Ala	Val	Glu	Trp	Glu	Ser	Asn	Gly	Gln	Pro	Glu	Asn	Asn	Tyr	Lys>
1260	ACC	ACG	CCT	CCC	GTG	CTG	GAC	TCC	GAC	GGC	TCC	TTC	TTC	CTC	TAC	AGC
	Thr	Thr	Pro	Pro	Val	Leu	Asp	Ser	Asp	Gly	Ser	Phe	Phe	Leu	Tyr	Ser>
1310	AAG	CTC	ACC	GTG	GAC	AAG	AGC	AGG	TGG	CAG	CAG	GGG	AAC	GTC	TTC	TCA
	Lys	Leu	Thr	Val	Asp	Lys	Ser	Arg	Trp	Gln	Gln	Gly	Asn	Val	Phe	Ser>
1350	TGC	TCC	GTG	ATG	CAT	GAG	GCT	CTG	CAC	AAC	CAC	TAC	ACG	CAG	AAG	AGC
	Cys	Ser	Val	Met	His	Glu	Ala	Leu	His	Asn	His	Tyr	Thr	Gln	Lys	Ser>
1400	CTC	TCC	CTG	TCT	CCG	GGT	AAA									
	Leu	Ser	Leu	Ser	Pro	Gly	Lys>									

FIG. 16 cont.

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FIG. 17.

Humanised 323/A3 (IgG_{4cys}) heavy chain cDNA sequence)

10	20	30	40	50											
CGTAAGCTTC	ACAGATCCTC	ACC	ATG	GGA	TGG	AGC	TGT	ATC	ATC	CTC	TTT	CTG			
			Met	Gly	Trp	Ser	Cys	Ile	Ile	Leu	Phe	Leu>			
60	70	80	90	100											
GTG	GCA	ACA	GCT	ACA	GGT	GTC	CAC	TCC	CAG	GTA	CAG	CTA	GTG	CAA	TCA
Val	Ala	Thr	Ala	Thr	Gly	Val	His	Ser	Xxx>	Val	Gln	Leu	Val	Gln	Ser>
110	120	130	140												
GGG	CCT	GAA	GTG	AAG	AAG	CCT	GGG	GCC	TCA	GTG	AAA	GTT	TCC	TGC	AAG
Gly	Pro	Glu	Val	Lys	Lys	Pro	Gly	Ala	Ser	Val	Lys	Val	Ser	Cys	Lys>
150	160	170	180	190											
GCT	TCT	GGC	TAC	ACC	TTC	ACC	AAC	TAT	GGA	ATG	AAC	TGG	GTA	AGG	CAG
Ala	Ser	Gly	Tyr	Thr	Phe	Thr	Asn	Tyr	Gly	Met	Asn	Trp	Val	Arg	Gln>
200	210	220	230	240											
GCG	CCT	GGA	CAG	GGG	CTT	GAG	TGG	ATG	GGG	TGG	ATA	AAC	ACC	TAC	ACT
Ala	Pro	Gly	Gln	Gly	Leu	Glu	Trp	Met	Gly	Trp	Ile	Asn	Thr	Tyr	Thr>
250	260	270	280	290											
GGA	GAG	CCA	ACA	TAT	GGT	GAA	GAT	TTC	AAG	GGA	CGG	TTT	GCA	TTC	TCT
Gly	Glu	Pro	Thr	Tyr	Gly	Glu	Asp	Phe	Lys	Gly	Arg	Phe	Ala	Phe	Ser>
300	310	320	330	340											
CTA	GAC	ACA	TCC	GCC	AGC	ACA	GCC	TAT	ATG	GAG	CTC	AGC	TCG	CTG	AGA
Leu	Asp	Thr	Ser	Ala	Ser	Thr	Ala	Tyr	Met	Glu	Leu	Ser	Ser	Leu	Arg>
350	360	370	380												
TCC	GAG	GAC	ACT	GCA	GTC	TAT	TTC	TGT	GCG	AGA	TTT	GGT	AAC	TAC	GTA
Ser	Glu	Asp	Thr	Ala	Val	Tyr	Phe	Cys	Ala	Arg	Phe	Gly	Asn	Tyr	Val>
390	400	410	420	430											
GAC	TAC	TGG	GGT	CAA	GGA	TCA	CTA	GTC	ACT	GTC	TCC	TCA	GCT	TCC	ACC
Asp	Tyr	Trp	Gly	Gln	Gly	Ser	Leu	Val	Thr	Val	Ser	Ser>	Ala	Ser	Thr>
440	450	460	470	480											
AAG	GGC	CCA	TCC	GTC	TTC	CCC	CTG	GCG	CCC	TGC	TCC	AGG	AGC	ACC	TCC
Lys	Gly	Pro	Ser	Val	Phe	Pro	Leu	Ala	Pro	Cys	Ser	Arg	Ser	Thr	Ser>

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490	500	510	520	530
GAG Glu	AGC Ser	ACA Thr	GCC Ala	GCC Ala
CTG Leu	GGC Gly	TGC Cys	CTG Leu	GTC Val
AAG Lys	GAC Asp	TAC Tyr	TTC Phe	CCC Pro
GAA Glu>				
540	550	560	570	580
CCG Pro	GTG Val	ACG Thr	GTG Val	TCG Ser
TGG Trp	AAC Asn	TCA Ser	GGC Gly	GCC Ala
CTG Leu	ACC Thr	AGC Ser	GGC Gly	GTG Val
CAC His>				
590	600	610	620	
ACC Thr	TTC Phe	CCG Pro	GCT Ala	GTG Val
CTA Leu	CAG Gln	TCC Ser	TCA Ser	GGA Gly
CTC Leu	TAC Tyr	TCC Ser	CTC Leu	AGC Ser
AGC Ser>				
630	640	650	660	670
GTG Val	GTG Val	ACC Thr	GTG Val	CCC Pro
TCC Ser	AGC Ser	AGC Ser	TTG Leu	GGC Gly
ACG Thr	AAG Lys	ACC Thr	TAC Tyr	ACC Thr
TGC Cys>				
680	690	700	710	720
AAC Asn	GTA Val	GAT Asp	CAC His	AAG Lys
CCC Pro	AGC Ser	AAC Asn	ACC Thr	AAG Lys
GTG Val	GAC Asp	AAG Lys	AGA Arg	GTT Val
GAG Glu>				
730	740	750	760	770
TCC Ser	AAA Lys	TAT Tyr	GGT Gly	CCC Pro
CCA Pro	TGC Cys	CCA Pro	CCG Pro	TGC Cys
CCT Pro	GCA Ala	CCT Pro	GAG Glu	TTC Phe
GCG Ala>				
780	790	800	810	820
GGG Gly	GCA Ala	CCA Pro	TCA Ser	GTC Val
TTC Phe	CTG Leu	TTC Phe	CCC Pro	CCA Pro
AAA Lys	CCC Pro	AAG Lys	GAC Asp	ACT Thr
CTC Leu>				
830	840	850	860	
ATG Met	ATC Ile	TCC Ser	CGG Arg	ACC Thr
CCT Pro	GAG Glu	GTC Val	ACG Thr	TGC Cys
GTG Val	GTG Val	GTG Val	GAC Asp	GTG Val
AGC Ser>				
870	880	890	900	910
CAG Gln	GAA Glu	GAC Asp	CCC Pro	GAG Glu
GTC Val	CAG Gln	TTC Phe	AAC Asn	TGG Trp
TAC Tyr	GTG Val	GAT Asp	GGC Gly	GTG Val
GAG Glu>				
920	930	940	950	960
GTG Val	CAT His	AAT Asn	GCC Ala	AAG Lys
ACA Thr	AAG Lys	CCG Pro	CGG Arg	GAG Glu
GAG Glu	CAG Gln	TTC Phe	AAC Asn	AGC Ser
ACG Thr>				

FIG. 17cont.

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970	TAC	CGT	GTG	GTC	980	AGC	GTC	CTC	990	ACC	GTC	CTG	CAC	1000	CAG	GAC	TGG	1010	CTG	ACC
	Tyr	Arg	Val	Val		Ser	Val	Leu		Thr	Val	Leu	His		Gln	Asp	Trp		Leu	Asn>
1020	GGC	AAG	GCG	TAC	1030	AAG	TGC	AAG	1040	GTC	TCC	AAC	AAA	1050	GGC	CTC	CCG	1060	TCC	TCC
	Gly	Lys	Ala	Tyr		Lys	Cys	Lys		Val	Ser	Asn	Lys		Gly	Leu	Pro		Ser	Ser>
1070	ATC	GAG	AAA	ACC	1080	ATC	TCC	AAA	1090	GCC	AAA	GGG	CAG	1100	CCC	CGA	GAG	CCA	CAG	
	Ile	Glu	Lys	Thr		Ile	Ser	Lys		Ala	Lys	Gly	Gln		Pro	Arg	Glu	Pro	Gln>	
1110	GTG	TAC	ACC	CTG	1120	CCC	CCA	TCC	1130	CAG	GAG	GAG	ATG	1140	ACC	AAG	AAC	CAG	GTC	
	Val	Tyr	Thr	Leu		Pro	Pro	Ser		Gln	Glu	Glu	Met		Thr	Lys	Asn	Gln	Val>	
1160	AGC	CTG	ACC	TGC	1170	CTG	GTC	AAA	1180	GGC	TTC	TAC	CCC	1190	AGC	GAC	ATC	GCC	GTG	
	Ser	Leu	Thr	Cys		Leu	Val	Lys		Gly	Phe	Tyr	Pro		Ser	Asp	Ile	Ala	Val>	
1210	GAG	TGG	GAG	AGC	1220	AAT	GGG	CAG	1230	CCG	GAG	AAC	AAC	1240	TAC	AAG	ACC	ACG	CCT	
	Glu	Trp	Glu	Ser		Asn	Gly	Gln		Pro	Glu	Asn	Asn		Tyr	Lys	Thr	Thr	Pro>	
1260	CCC	GTG	CTG	GAC	1270	TCC	GAC	GGC	1280	TCC	TTC	TTC	CTC	1290	TAC	AGC	AGG	CTA	ACC	
	Pro	Val	Leu	Asp		Ser	Asp	Gly		Ser	Phe	Phe	Leu		Tyr	Ser	Arg	Leu	Thr>	
1310	GTG	GAC	AAG	AGC	1320	AGG	TGG	CAG	1330	GAG	GGG	AAT	GTC	1340	TTC	TCA	TGC	TCC	GTG	
	Val	Asp	Lys	Ser		Arg	Trp	Gln		Glu	Gly	Asn	Val		Phe	Ser	Cys	Ser	Val>	
1350	ATG	CAT	GAG	GCT	1360	CTG	CAC	AAC	1370	CAC	TAC	ACA	CAG	1380	AAG	AGC	CTC	TGC	CTG	
	Met	His	Glu	Ala		Leu	His	Asn		His	Tyr	Thr	Gln		Lys	Ser	Leu	Cys	Leu>	
1400	TCT	CTG	GGT	AAA	1410	T	GAGAATTC													
	Ser	Leu	Gly	Lys>																

FIG. 17cont.

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FIG. 18.

Humanised 323/A3 (IgG_{2cys}) heavy chain cDNA sequence

```

      10      20      30      40      50      60
ATGGATTGGC  TGTGGAAC TT GCTATTCCTG  ATGGCAGCTG  CCCAAAGTAT  CCAAGCA  CAG
TACCTAACCG  ACACCTTGAA  CGATAAGGAC  TACCGTCGAC  GGGTTTCATA  GGTTCGT  GTC
                                           Gln>

      70      80      90      100
ATC  CAG  TTG  GTG  CAG  TCT  GGA  CCT  GAA  CTG  AAG  AAG  CCT  GGA  GAG  ACA
TAG  GTC  AAC  CAC  GTC  AGA  CCT  CTT  GAC  GAC  TTC  TTC  GGA  CCT  CTC  TGT
Ile  Gln  Leu  Val  Gln  Ser  Gly  Pro  Glu  Leu  Lys  Lys  Pro  Gly  Glu  Thr>

110      120      130      140      150
GTC  AAG  ATC  TCC  TGC  AAG  GCT  TCT  GGA  TAT  ACC  TTC  ACA  AAC  TAT  GGA
CAG  TTC  TAG  AGG  ACG  TTC  CGA  AGA  CCT  ATA  TGG  AAG  TGT  TTG  ATA  CCT
Val  Lys  Ile  Ser  Cys  Lys  Ala  Ser  Gly  Tyr  Thr  Phe  Thr  Asn  Tyr  Gly>

160      170      180      190      200
ATG  AAC  TGG  GTG  AGG  CAG  GCT  TCA  GGA  GAG  GGT  TTA  AAG  TGG  ATG  GGC
TAC  TTG  ACC  CAC  TCC  GTC  CGA  AGT  CCT  CTC  CCA  AAT  TTC  ACC  TAC  CCG
Met  Asn  Trp  Val  Arg  Gln  Ala  Ser  Gly  Glu  Gly  Leu  Lys  Trp  Met  Gly>

210      220      230      240      250
TGG  ATA  AAC  ACC  TAC  ACT  GGA  GAG  CCA  ACA  TAT  GGT  GAA  GAT  TTC  AAG
ACC  TAT  TTG  TGG  ATG  TGA  CCT  CTC  GGT  TGT  ATA  CCA  CTT  CTA  AAG  TTC
Trp  Ile  Asn  Thr  Tyr  Thr  Gly  Glu  Pro  Thr  Tyr  Gly  Glu  Asp  Phe  Lys>

260      270      280      290      300
GGA  CGG  TTT  GCC  TTC  TCT  TTG  GAA  ACC  TCT  GCC  AGC  ACT  GCC  TAT  TTG
CCT  GCC  AAA  CGG  AAG  AGA  AAC  CTT  TGG  AGA  CGG  TCG  TGA  CGG  ATA  AAC
Gly  Arg  Phe  Ala  Phe  Ser  Leu  Glu  Thr  Ser  Ala  Ser  Thr  Ala  Tyr  Leu>

310      320      330      340
CAG  ATC  AAC  AAC  CTC  AAA  AAT  GAA  GAC  ACG  GCT  ACA  TAT  TTC  TGT  GCA
GTC  TAG  TTG  TTG  GAG  TTT  TTA  CTT  CTG  TGC  CGA  TGT  ATA  AAG  ACA  CGT
Gln  Ile  Asn  Asn  Leu  Lys  Asn  Glu  Asp  Thr  Ala  Thr  Tyr  Phe  Cys  Ala>

350      360      370      380      390
AGA  TTT  GGT  AAC  TAC  GTA  GAC  TAC  TGG  GGC  CAA  GGC  ACC  ACT  CTC  ACA
TCT  AAA  CCA  CCA  ATG  CAT  CTG  ATG  ACC  CCG  GTT  CCG  TGG  TGA  GAG  TGT
Arg  Phe  Gly  Asn  Tyr  Val  Asp  Tyr  Trp  Gly  Gln  Gly  Thr  Thr  Leu  Thr>

400      410      420      430      440
GTC  TCC  TCA  GCC  TCC  ACC  AAG  GGC  CCA  TCG  GTC  TTC  CCC  CTG  GCG  CCC
CAG  AGG  AGT  CGG  AGG  TGG  TTC  CCG  GGT  AGC  CAG  AAG  GGG  GAC  CGC  GGG
Val  Ser  Ser>  Ala  Ser  Thr  Lys  Gly  Pro  Ser  Val  Phe  Pro  Leu  Ala  Pro>

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450	TGC	TCC	AGG	AGC	ACC	TCC	GAG	AGC	ACA	GCG	GCC	CTG	GGC	TGC	CTG	GTC
	ACG	AGG	TCC	TCG	TGG	AGG	CTC	TCG	TGT	CGC	CGG	GAC	CCG	ACG	GAC	CAG
	Cys	Ser	Arg	Ser	Thr	Ser	Glu	Ser	Thr	Ala	Ala	Leu	Gly	Cys	Leu	Val>
500	AAG	GAC	TAC	TTC	CCC	GAA	CCG	GTG	ACG	GTG	TCG	TGG	AAC	TCA	GGC	GCT
	TTC	CTG	ATG	AAG	GGG	CTT	GGC	CAC	TGC	CAC	AGC	ACC	TTG	AGT	CCG	CGA
	Lys	Asp	Tyr	Phe	Pro	Glu	Pro	Val	Thr	Val	Ser	Trp	Asn	Ser	Gly	Ala>
550	CTG	ACC	AGC	GGC	GTG	CAC	ACC	TTC	CCA	GCT	GTC	CTA	CAG	TCC	TCA	GGA
	GAC	TGG	TCG	CCG	CAC	GTG	TGG	AAG	GGT	CGA	CAG	GAT	GTC	AGG	AGT	CCT
	Leu	Thr	Ser	Gly	Val	His	Thr	Phe	Pro	Ala	Val	Leu	Gln	Ser	Ser	Gly>
590	CTC	TAC	TCC	CTC	AGC	AGC	GTG	GTG	ACC	GTG	CCC	TCC	AGC	AAC	TTC	GGC
	GAG	ATG	AGG	GAG	TCG	TCG	CAC	CAC	TGG	CAC	GGG	AGG	TCG	TTG	AAG	CCG
	Leu	Tyr	Ser	Leu	Ser	Ser	Val	Val	Thr	Val	Pro	Ser	Ser	Asn	Phe	Gly>
640	ACC	CAG	ACC	TAC	ACC	TGC	AAC	GTA	GAT	CAC	AAG	CCC	AGC	AAC	ACC	AAG
	TGG	GTC	TGG	ATG	TGG	ACG	TTG	CAT	CTA	GTG	TTC	GGG	TCG	TTG	TGG	TTC
	Thr	Gln	Thr	Tyr	Thr	Cys	Asn	Val	Asp	His	Lys	Pro	Ser	Asn	Thr	Lys>
690	GTG	GAC	AAG	ACA	GTT	GAG	CGC	AAA	TGT	TGT	GTC	GAG	TGC	CCA	CCG	TGC
	CAC	CTG	TTC	TGT	CAA	CTC	GCG	TTT	ACA	ACA	CAG	CTC	ACG	GGT	GGC	ACG
	Val	Asp	Lys	Thr	Val	Glu	Arg	Lys	Cys	Cys	Val	Glu	Cys	Pro	Pro	Cys>
740	CCA	GCA	CCA	CCT	GTG	GCA	GGA	CCG	TCA	GTC	TTC	CTC	TTC	CCC	CCA	AAA
	GGT	CGT	GGT	GGA	CAC	CGT	CCT	GGC	AGT	CAG	AAG	GAG	AAG	GGG	GGT	TTT
	Pro	Ala	Pro	Pro	Val	Ala	Gly	Pro	Ser	Val	Phe	Leu	Phe	Pro	Pro	Lys>
790	CCC	AAG	GAC	ACC	CTC	ATG	ATC	TCC	CGG	ACC	CCT	GAG	GTC	ACG	TGC	GTG
	GGG	TTC	CTG	TGG	GAG	TAC	TAG	AGG	GCC	TGG	GGA	CTC	CAG	TGC	ACG	CAC
	Pro	Lys	Asp	Thr	Leu	Met	Ile	Ser	Arg	Thr	Pro	Glu	Val	Thr	Cys	Val>
830	GTG	GTG	GAC	GTG	AGC	CAC	GAA	GAC	CCC	GAG	GTC	CAG	TTC	AAC	TGG	TAC
	CAC	CAC	CTG	CAC	TCG	GTG	CTT	CTG	GGG	CTC	CAG	GTC	AAG	TTG	ACC	ATG
	Val	Val	Asp	Val	Ser	His	Glu	Asp	Pro	Glu	Val	Gln	Phe	Asn	Trp	Tyr>

FIG. 18cont.

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880	GTG CAC Val	GAC CTG Asp	GGC CCG Gly	GTG CAC Val	890	GAG CTC Glu	GTG CAC Val	CAT GTA His	900	AAT TTA Asn	GCC CGG Ala	AAG TTC Lys	ACA TGT Thr	910	AAG TTC Lys	CCA GGT Pro	CGG GCC Arg	920	GAG CTC Glu	GAG CTC Glu>
930	CAG GTC Gln	TTC AAG Phe	AAC TTG Asn	AGC TCG Ser	940	ACG TGC Thr	TTC AAG Phe	CGT GCA Arg	950	GTG CAC Val	GTC CAG Val	AGC TCG Ser	GTC CAG Val	960	CTC GAG Leu	ACC TGG Thr	GTT CAA Val	970	GTG CAC Val	CAC GTG His>
980	CAG GTC Gln	GAC CTG Asp	TGG ACC Trp	CTG GAC Leu	990	AAC TTG Asn	GGC CCG Gly	AAG TTC Lys	1000	GAG CTC Glu	TAC ATG Tyr	AAG TTC Lys	TGC ACG Cys	1010	AAG TTC Lys	GTC CAG Val	TCC AGG Ser	1020	AAC TTG Asn	AAA TTT Lys>
1030	GGC CCG Gly	CTC GAG Leu	CCA GGT Pro	GCC CGG Ala	1040	CCC GGG Pro	ATC TAG Ile	GAG CTC Glu	1050	AAA TTT Lys	ACC TGG Thr	ATC TAG Ile	TCC AGG Ser	1060	AAA TTT Lys	ACC TGG Thr	AAA TTT Lys	GGG CCC Gly	CAG GTC Gln>	
1070	CCC GGG Pro	CGA GCT Arg	GAA CTT Glu	1080	CCA GGT Pro	CAG GTC Val	GTG CAC Val	TAC ATG Tyr	1090	ACC TGG Thr	CTG GAC Leu	CCC GGG Pro	CCA GGT Pro	1100	TCC AGG Ser	CGG GCC Arg	GAG CTC Glu	1110	GAG CTC Glu	ATG TAC Met>
1120	ACC TGG Thr	AAG TTC Lys	AAC TTG Asn	1130	CAG GTC Gln	GTC CAG Val	AGC TCG Ser	CTG GAC Leu	1140	ACC TGG Thr	TGC ACG Cys	CTG GAC Leu	GTC CAG Val	1150	AAA TTT Lys	GGC CCG Gly	TTC AAG Phe	1160	TAC ATG Tyr	CCC GGG Pro>
1170	AGC TCG Ser	GAC CTG Asp	ATC TAG Ile	1180	GCC CGG Ala	GTG CAC Val	GAG CTC Glu	TGG ACC Trp	1190	GAG CTC Glu	AGC TCG Ser	AAT TTA Asn	GGG CCC Gly	1200	CAG GTC Gln	CCG GGC Pro	GAG CTC Glu	1210	AAC TTG Asn	AAC TTG Asn>
1220	TAC ATG Tyr	AAG TTC Lys	ACC TGG Thr	1230	ACA TGT Thr	CCT GGA Pro	CCC GGG Pro	ATG TAC Met	1240	CTG GAC Leu	GAC CTG Asp	TCC AGG Ser	GAC CTG Asp	1250	GGC CCG Gly	TCC AGG Ser	TTC AAG Phe	1260	TTC AAG Phe	CTC GAG Leu>

FIG. 18 cont.

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1270				1280				1290				1300					
TAC	AGC	AAG	CTC	ACC	GTG	GAC	AAG	AGC	AGG	TGG	CAG	CAG	GGG	AAC	GTC		
ATG	TCG	TTC	GAG	TGG	CAC	CTG	TTC	TCG	TCC	ACC	GTC	GTC	CCC	TTG	CAG		
Tyr	Ser	Lys	Leu	Thr	Val	Asp	Lys	Ser	Arg	Trp	Gln	Gln	Gly	Asn	Val>		
1310				1320				1330				1340				1350	
TTC	TCA	TGC	TCC	GTG	ATG	CAT	GAG	GCT	CTG	CAC	AAC	CAC	TAC	ACA	CAG		
AAG	AGT	ACG	AGG	CAC	TAC	GTA	CTC	CGA	GAC	GTG	TTG	GTG	ATG	TGT	GTC		
Phe	Ser	Cys	Ser	Val	Met	His	Glu	Ala	Leu	His	Asn	His	Tyr	Thr	Gln>		
1360				1370				1380				1390					
AAG	AGC	CTC	TGC	CTG	TCT	CTG	GGT	AAA	AGAGAAT		TC						
TTC	TCG	GAG	ACG	GAC	AGA	GAC	CCA	TTT	ACTCTTA		AG						
Lys	Ser	Leu	Cys	Leu	Ser	Leu	Gly	Lys>									

FIG. 18cont.

SEQUENCE LISTING

<110> Glaxo Group Limited
Knick, Vincent C
Stimmel, Julie B
Thurmond, Linda M

<120> Antibody combination

<130> PQ3513

<140>

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<150> GB 9816280.3

<151> 1998-07-27

<160> 16

<170> PatentIn Ver. 2.1

<210> 1

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Met Gly Trp Ser Cys Ile Ile Leu Phe Leu

gta gca aca gct aca ggt gtc cac tcc gat att gtg atg act cag tct 101
Val Ala Thr Ala Thr Gly Val His Ser Asp Ile Val Met Thr Gln Ser

15

20

25

cca ctc tcc ctg ccc gtc acc cct gga gag ccg gcc tcc atc tcc tgt 149
Pro Leu Ser Leu Pro Val Thr Pro Gly Glu Pro Ala Ser Ile Ser Cys

30

35

40

agg tct agt aag aat ctc ctg cat agt aat ggc atc act tat ttg tat 197
Arg Ser Ser Lys Asn Leu Leu His Ser Asn Gly Ile Thr Tyr Leu Tyr

45

50

55

tgg tac ctg cag aag cca ggg cag tct cca cag ctc ctg atc tat cag 245
Trp Tyr Leu Gln Lys Pro Gly Gln Ser Pro Gln Leu Leu Ile Tyr Gln

60

65

70

atg tcc aac ctt gcc tca ggg gtc cct gac agg ttc agt agc agt gga 293
Met Ser Asn Leu Ala Ser Gly Val Pro Asp Arg Phe Ser Ser Ser Gly

75

80

85

90

tca ggc aca gat ttt aca ctg aaa atc agc aga gtg gag gct gag gat 341
Ser Gly Thr Asp Phe Thr Leu Lys Ile Ser Arg Val Glu Ala Glu Asp

95

100

105

gtt ggg gtt tat tac tgt gct caa aat cta gag att cct cgg acg ttc 389
Val Gly Val Tyr Tyr Cys Ala Gln Asn Leu Glu Ile Pro Arg Thr Phe

110

115

120

ggc caa ggg acc aag gtg gag atc aaa cgt acg gtg gct gca cca tct 437
Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala Pro Ser

125

130

135

gtc ttc atc ttc ccg cca tct gat gag cag ttg aaa tct gga act gcc 485
Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr Ala

140

145

150

tct gtt gtg tgc ctg ctg aat aac ttc tat ccc aga gag gcc aaa gta 533
 Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys Val
 155 160 165 170

cag tgg aag gtg gat aac gcc ctc caa tcg ggt aac tcc cag gag agt 581
 Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu Ser
 175 180 185

gtc aca gag cag gac agc aag gac agc acc tac agc ctc agc agc acc 629
 Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser Thr
 190 195 200

ctg acg ctg agc aaa gca gac tac gag aaa cac aaa gtc tac gcc tgc 677
 Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr Ala Cys
 205 210 215

gaa gtc acc cat cag ggc ctg agc tcg ccc gtc aca aag agc ttc aac 725
 Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser Phe Asn
 220 225 230

agg gga gag tgt tag 740
 Arg Gly Glu Cys
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<212> PRT

<213> Artificial Sequence

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Val His Ser Asp Ile Val Met Thr Gln Ser Pro Leu Ser Leu Pro Val
 20 25 30

Thr Pro Gly Glu Pro Ala Ser Ile Ser Cys Arg Ser Ser Lys Asn Leu
35 40 45

Leu His Ser Asn Gly Ile Thr Tyr Leu Tyr Trp Tyr Leu Gln Lys Pro
50 55 60

Gly Gln Ser Pro Gln Leu Leu Ile Tyr Gln Met Ser Asn Leu Ala Ser
65 70 75 80

Gly Val Pro Asp Arg Phe Ser Ser Ser Gly Ser Gly Thr Asp Phe Thr
85 90 95

Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys
100 105 110

Ala Gln Asn Leu Glu Ile Pro Arg Thr Phe Gly Gln Gly Thr Lys Val
115 120 125

Glu Ile Lys Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro
130 135 140

Ser Asp Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu
145 150 155 160

Asn Asn Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn
165 170 175

Ala Leu Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser
180 185 190

Lys Asp Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala
195 200 205

Asp Tyr Glu Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly
210 215 220

Leu Ser Ser Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
225 230 235

<210> 3

<211> 740

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic
sequence

<400> 3

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ctaacactct cccctgttga agctctttgt gacgggagag ctcaggccct gatgggtgac 60
ttcgcaggcg tagactttgt gtttctcgta gtctgctttg ctcagcgta ggggtgctgct 120
gaggctgtag gtgctgtcct tgctgtcctg ctctgtgaca ctctcctggg agttaccgga 180
ttggagggcg ttatccacct tccactgtac tttggcctct ctgggataga agttattcag 240
caggcacaca acagaggcag ttccagattt caactgctca tcagatggcg ggaagatgaa 300
gacagatggt gcagccaccg tacgtttgat ctccaccttg gtcccttggc cgaacgtccg 360
aggaatctct agattttgag cacagtaata aacccaaca tcctcagcct ccactctgct 420
gattttcagt gtaaaatctg tgcctgatcc actgctactg aacctgtcag ggaccctga 480
ggcaagggtg gacatctgat agatcaggag ctgtggagac tgccctggct tctgcaggta 540
ccaatacaaa taagtgtatg cattactatg caggagattc ttactagacc tacaggagat 600
ggaggccggc tctccagggg tgacgggcag ggagagtggg gactgagtca tcacaatatc 660
ggagtggaca cctgtagctg ttgctaccaa gaagaggatg atacagctcc atcccatggt 720
gaggtcctgt gaagcttacg                                     740
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<210> 4

<211> 1418

<212> DNA

<213> Artificial Sequence

<220>

<221> CDS

<222> (24)..(1418)

<220>

<223> Description of Artificial Sequence: Synthetic
sequence

<400> 4

cgtaagcttc acagatcctc acc atg gga tgg agc tgt atc atc ctc ttt ctg 53

Met Gly Trp Ser Cys Ile Ile Leu Phe Leu

1

5

10

gtg gca aca gct aca ggt gtc cac tcc cag gta cag cta gtg caa tca 101

Val Ala Thr Ala Thr Gly Val His Ser Gln Val Gln Leu Val Gln Ser

15

20

25

ggg cct gaa gtg aag aag cct ggg gcc tca gtg aaa gtt tcc tgc aag 149

Gly Pro Glu Val Lys Lys Pro Gly Ala Ser Val Lys Val Ser Cys Lys

30

35

40

gct tct ggc tac acc ttc acc aac tat gga atg aac tgg gta agg cag 197

Ala Ser Gly Tyr Thr Phe Thr Asn Tyr Gly Met Asn Trp Val Arg Gln

45

50

55

gcg cct gga cag ggg ctt gag tgg atg ggg tgg ata aac acc tac act 245

Ala Pro Gly Gln Gly Leu Glu Trp Met Gly Trp Ile Asn Thr Tyr Thr

60

65

70

gga gag cca aca tat ggt gaa gat ttc aag gga cgg ttt gca ttc tct 293

Gly Glu Pro Thr Tyr Gly Glu Asp Phe Lys Gly Arg Phe Ala Phe Ser

75

80

85

90

cta gac aca tcc gcc agc aca gcc tat atg gag ctc agc tcg ctg aga 341

Leu Asp Thr Ser Ala Ser Thr Ala Tyr Met Glu Leu Ser Ser Leu Arg

95

100

105

tcc gag gac act gca gtc tat ttc tgt gcg aga ttt ggt aac tac gta 389

Ser Glu Asp Thr Ala Val Tyr Phe Cys Ala Arg Phe Gly Asn Tyr Val

110

115

120

gac tac tgg ggt caa gga tca cta gtc act gtc tcc tca gcc tcc acc 437
 Asp Tyr Trp Gly Gln Gly Ser Leu Val Thr Val Ser Ser Ala Ser Thr
 125 130 135

aag ggc cca tcg gtc ttc ccc ctg gca ccc tcc tcc aag agc acc tct 485
 Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser
 140 145 150

ggg ggc aca gcg gcc ctg ggc tgc ctg gtc aag gac tac ttc ccc gaa 533
 Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu
 155 160 165 170

ccg gtg acg gtg tcg tgg aac tca ggc gcc ctg acc agc ggc gtg cac 581
 Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His
 175 180 185

acc ttc ccg gct gtc cta cag tcc tca gga ctc tac tcc ctc agc agc 629
 Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser
 190 195 200

gtg gtg acc gtg ccc tcc agc agc ttg ggc acc cag acc tac atc tgc 677
 Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys
 205 210 215

aac gtg aat cac aag ccc agc aac acc aag gtg gac aag aaa gtt gag 725
 Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu
 220 225 230

ccc aaa tct tgt gac aaa act cac aca tgc cca ccg tgc cca gca cct 773
 Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro
 235 240 245 250

gaa ctc ctg ggg gga ccg tca gtc ttc ctc ttc ccc cca aaa ccc aag 821
 Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys
 255 260 265

gac acc ctc atg atc tcc cgg acc cct gag gtc aca tgc gtg gtg gtg 869
 Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val
 270 275 280

gac gtg agc cac gaa gac cct gag gtc aag ttc aac tgg tac gtg gac 917
 Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp
 285 290 295

ggc gtg gag gtg cat aat gcc aag aca aag ccg cgg gag gag cag tac 965
 Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr
 300 305 310

aac agc acg tac cgt gtg gtc agc gtc ctc acc gtc ctg cac cag gac 1013
 Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp
 315 320 325 330

tgg ctg aat ggc aag gag tac aag tgc aag gtc tcc aac aaa gcc ctc 1061
 Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu
 335 340 345

cca gcc ccc atc gag aaa acc atc tcc aaa gcc aaa ggg cag ccc cga 1109
 Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg
 350 355 360

gaa cca cag gtg tac acc ctg ccc cca tcc cgg gat gag ctg acc aag 1157
 Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys
 365 370 375

aac cag gtc agc ctg acc tgc ctg gtc aaa ggc ttc tat ccc agc gac 1205
 Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp
 380 385 390

atc gcc gtg gag tgg gag agc aat ggg cag ccg gag aac aac tac aag 1253
 Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys
 395 400 405 410

acc acg cct ccc gtg ctg gac tcc gac ggc tcc ttc ttc ctc tac agc 1301
 Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser
 415 420 425

aag ctc acc gtg gac aag agc agg tgg cag cag ggg aac gtc ttc tca 1349
 Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser
 430 435 440

tgc tcc gtg atg cat gag gct ctg cac aac cac tac acg cag aag agc 1397
 Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser
 445 450 455

ctc tcc ctg tct ccg ggt aaa 1418
 Leu Ser Leu Ser Pro Gly Lys
 460 465

<210> 5

<211> 465

<212> PRT

<213> Artificial Sequence

<223> Description of Artificial Sequence: Synthetic
 sequence

<400> 5

Met Gly Trp Ser Cys Ile Ile Leu Phe Leu Val Ala Thr Ala Thr Gly
 1 5 10 15

Val His Ser Gln Val Gln Leu Val Gln Ser Gly Pro Glu Val Lys Lys
 20 25 30

Pro Gly Ala Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe
 35 40 45

Thr Asn Tyr Gly Met Asn Trp Val Arg Gln Ala Pro Gly Gln Gly Leu
 50 55 60

10

Glu Trp Met Gly Trp Ile Asn Thr Tyr Thr Gly Glu Pro Thr Tyr Gly
65 70 75 80

Glu Asp Phe Lys Gly Arg Phe Ala Phe Ser Leu Asp Thr Ser Ala Ser
85 90 95

Thr Ala Tyr Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val
100 105 110

Tyr Phe Cys Ala Arg Phe Gly Asn Tyr Val Asp Tyr Trp Gly Gln Gly
115 120 125

Ser Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe
130 135 140

Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu
145 150 155 160

Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp
165 170 175

Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu
180 185 190

Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser
195 200 205

Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro
210 215 220

Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys
225 230 235 240

Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro
245 250 255

Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser
260 265 270

Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp
 275 280 285

Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn
 290 295 300

Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val
 305 310 315 320

Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu
 325 330 335

Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys
 340 345 350

Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr
 355 360 365

Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr
 370 375 380

Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu
 385 390 395 400

Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu
 405 410 415

Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys
 420 425 430

Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu
 435 440 445

Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly
 450 455 460

Lys

465

<210> 6

<211> 1418

<212> DNA

<213> Artificial Sequence

<220>

<221> CDS

<222> (24)..(1412)

<220>

<223> Description of Artificial Sequence: Synthetic
sequence

<400> 6

cgtaagcttc acagatcctc acc atg gga tgg agc tgt atc atc ctc ttt ctg 53

Met Gly Trp Ser Cys Ile Ile Leu Phe Leu

1

5

10

gtg gca aca gct aca ggt gtc cac tcc cag gta cag cta gtg caa tca 101

Val Ala Thr Ala Thr Gly Val His Ser Gln Val Gln Leu Val Gln Ser

15

20

25

ggg cct gaa gtg aag aag cct ggg gcc tca gtg aaa gtt tcc tgc aag 149

Gly Pro Glu Val Lys Lys Pro Gly Ala Ser Val Lys Val Ser Cys Lys

30

35

40

gct tct ggc tac acc ttc acc aac tat gga atg aac tgg gta agg cag 197

Ala Ser Gly Tyr Thr Phe Thr Asn Tyr Gly Met Asn Trp Val Arg Gln

45

50

55

gcg cct gga cag ggg ctt gag tgg atg ggg tgg ata aac acc tac act 245

Ala Pro Gly Gln Gly Leu Glu Trp Met Gly Trp Ile Asn Thr Tyr Thr

60

65

70

gga gag cca aca tat ggt gaa gat ttc aag gga cgg ttt gca ttc tct 293

Gly Glu Pro Thr Tyr Gly Glu Asp Phe Lys Gly Arg Phe Ala Phe Ser

75

80

85

90

13

cta gac aca tcc gcc agc aca gcc tat atg gag ctc agc tcg ctg aga 341
 Leu Asp Thr Ser Ala Ser Thr Ala Tyr Met Glu Leu Ser Ser Leu Arg
 95 100 105

tcc gag gac act gca gtc tat ttc tgt gcg aga ttt ggt aac tac gta 389
 Ser Glu Asp Thr Ala Val Tyr Phe Cys Ala Arg Phe Gly Asn Tyr Val
 110 115 120

gac tac tgg ggt caa gga tca cta gtc act gtc tcc tca gct tcc acc 437
 Asp Tyr Trp Gly Gln Gly Ser Leu Val Thr Val Ser Ser Ala Ser Thr
 125 130 135

aag ggc cca tcc gtc ttc ccc ctg gcg ccc tgc tcc agg agc acc tcc 485
 Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser
 140 145 150

gag agc aca gcc gcc ctg ggc tgc ctg gtc aag gac tac ttc ccc gaa 533
 Glu Ser Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu
 155 160 165 170

ccg gtg acg gtg tcg tgg aac tca ggc gcc ctg acc agc ggc gtg cac 581
 Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His
 175 180 185

acc ttc ccg gct gtc cta cag tcc tca gga ctc tac tcc ctc agc agc 629
 Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser
 190 195 200

gtg gtg acc gtg ccc tcc agc agc ttg ggc acg aag acc tac acc tgc 677
 Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Lys Thr Tyr Thr Cys
 205 210 215

aac gta gat cac aag ccc agc aac acc aag gtg gac aag aga gtt gag 725
 Asn Val Asp His Lys Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu
 220 225 230

tcc aaa tat ggt ccc cca .tgc cca ccg tgc cct gca cct gag ttc gcg 773
 Ser Lys Tyr Gly Pro Pro Cys Pro Pro Cys Pro Ala Pro Glu Phe Ala
 235 240 245 250

ggg gca cca tca gtc ttc ctg ttc ccc cca aaa ccc aag gac act ctc 821
 Gly Ala Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu
 255 260 265

atg atc tcc cgg acc cct gag gtc acg tgc gtg gtg gtg gac gtg agc 869
 Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser
 270 275 280

cag gaa gac ccc gag gtc cag ttc aac tgg tac gtg gat ggc gtg gag 917
 Gln Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Val Glu
 285 290 295

gtg cat aat gcc aag aca aag ccg cgg gag gag cag ttc aac agc acg 965
 Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr
 300 305 310

tac cgt gtg gtc agc gtc ctc acc gtc ctg cac cag gac tgg ctg acc 1013
 Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Thr
 315 320 325 330

ggc aag gcg tac aag tgc aag gtc tcc aac aaa ggc ctc ccg tcc tcc 1061
 Gly Lys Ala Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu Pro Ser Ser
 335 340 345

atc gag aaa acc atc tcc aaa gcc aaa ggg cag ccc cga gag cca cag 1109
 Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln
 350 355 360

gtg tac acc ctg ccc cca tcc cag gag gag atg acc aag aac cag gtc 1157
 Val Tyr Thr Leu Pro Pro Ser Gln Glu Glu Met Thr Lys Asn Gln Val
 365 370 375

15

agc ctg acc tgc ctg gtc aaa ggc ttc tac ccc agc gac atc gcc gtg 1205
 Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val
 380 385 390

gag tgg gag agc aat ggg cag ccg gag aac aac tac aag acc acg cct 1253
 Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro
 395 400 405 410

ccc gtg ctg gac tcc gac ggc tcc ttc ttc ctc tac agc agg cta acc 1301
 Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Arg Leu Thr
 415 420 425

gtg gac aag agc agg tgg cag gag ggg aat gtc ttc tca tgc tcc gtg 1349
 Val Asp Lys Ser Arg Trp Gln Glu Gly Asn Val Phe Ser Cys Ser Val
 430 435 440

atg cat gag gct ctg cac aac cac tac aca cag aag agc ctc tgc ctg 1397
 Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Cys Leu
 445 450 455

tct ctg ggt aaa tga gaattc 1418
 Ser Leu Gly Lys
 460

<210> 7

<211> 462

<212> PRT

<213> Artificial Sequence

<223> Description of Artificial Sequence: Synthetic
 sequence

<400> 7

Met Gly Trp Ser Cys Ile Ile Leu Phe Leu Val Ala Thr Ala Thr Gly
 1 5 10 15

Val His Ser Gln Val Gln Leu Val Gln Ser Gly Pro Glu Val Lys Lys
 20 25 30

16

Pro Gly Ala Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe
35 40 45

Thr Asn Tyr Gly Met Asn Trp Val Arg Gln Ala Pro Gly Gln Gly Leu
50 55 60

Glu Trp Met Gly Trp Ile Asn Thr Tyr Thr Gly Glu Pro Thr Tyr Gly
65 70 75 80

Glu Asp Phe Lys Gly Arg Phe Ala Phe Ser Leu Asp Thr Ser Ala Ser
85 90 95

Thr Ala Tyr Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val
100 105 110

Tyr Phe Cys Ala Arg Phe Gly Asn Tyr Val Asp Tyr Trp Gly Gln Gly
115 120 125

Ser Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe
130 135 140

Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser Glu Ser Thr Ala Ala Leu
145 150 155 160

Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp
165 170 175

Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu
180 185 190

Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser
195 200 205

Ser Ser Leu Gly Thr Lys Thr Tyr Thr Cys Asn Val Asp His Lys Pro
210 215 220

Ser Asn Thr Lys Val Asp Lys Arg Val Glu Ser Lys Tyr Gly Pro Pro
225 230 235 240

Cys Pro Pro Cys Pro Ala Pro Glu Phe Ala Gly Ala Pro Ser Val Phe
 245 250 255

Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro
 260 265 270

Glu Val Thr Cys Val Val Val Asp Val Ser Gln Glu Asp Pro Glu Val
 275 280 285

Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr
 290 295 300

Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Tyr Arg Val Val Ser Val
 305 310 315 320

Leu Thr Val Leu His Gln Asp Trp Leu Thr Gly Lys Ala Tyr Lys Cys
 325 330 335

Lys Val Ser Asn Lys Gly Leu Pro Ser Ser Ile Glu Lys Thr Ile Ser
 340 345 350

Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro
 355 360 365

Ser Gln Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val
 370 375 380

Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly
 385 390 395 400

Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp
 405 410 415

Gly Ser Phe Phe Leu Tyr Ser Arg Leu Thr Val Asp Lys Ser Arg Trp
 420 425 430

Gln Glu Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His
 435 440 445

Asn His Tyr Thr Gln Lys Ser Leu Cys Leu Ser Leu Gly Lys
 450 455 460

<210> 8

<211> 1392

<212> DNA

<213> Artificial Sequence

<220>

<221> CDS

<222> (58)..(1386)

<220>

<223> Description of Artificial Sequence: Synthetic
 sequence

<400> 8.

atggattggc tgtggaactt gctattcctg atggcagctg cccaaagtat ccaagca 57

cag atc cag ttg gtg cag tct gga cct gaa ctg aag aag cct gga gag 105

Gln Ile Gln Leu Val Gln Ser Gly Pro Glu Leu Lys Lys Pro Gly Glu

1 5 10 15

aca gtc aag atc tcc tgc aag gct tct gga tat acc ttc aca aac tat 153

Thr Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asn Tyr

20 25 30

gga atg aac tgg gtg agg cag gct tca gga gag ggt tta aag tgg atg 201

Gly Met Asn Trp Val Arg Gln Ala Ser Gly Glu Gly Leu Lys Trp Met

35 40 45

ggc tgg ata aac acc tac act gga gag cca aca tat ggt gaa gat ttc 249

Gly Trp Ile Asn Thr Tyr Thr Gly Glu Pro Thr Tyr Gly Glu Asp Phe

50 55 60

aag gga cgg ttt gcc ttc tct ttg gaa acc tct gcc agc act gcc tat 297
 Lys Gly Arg Phe Ala Phe Ser Leu Glu Thr Ser Ala Ser Thr Ala Tyr
 65 70 75 80

ttg cag atc aac aac ctc aaa aat gaa gac acg gct aca tat ttc tgt 345
 Leu Gln Ile Asn Asn Leu Lys Asn Glu Asp Thr Ala Thr Tyr Phe Cys
 85 90 95

gca aga ttt ggt aac tac gta gac tac tgg ggc caa ggc acc act ctc 393
 Ala Arg Phe Gly Asn Tyr Val Asp Tyr Trp Gly Gln Gly Thr Thr Leu
 100 105 110

aca gtc tcc tca gcc tcc acc aag ggc cca tcg gtc ttc ccc ctg gcg 441
 Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala
 115 120 125

ccc tgc tcc agg agc acc tcc gag agc aca gcg gcc ctg ggc tgc ctg 489
 Pro Cys Ser Arg Ser Thr Ser Glu Ser Thr Ala Ala Leu Gly Cys Leu
 130 135 140

gtc aag gac tac ttc ccc gaa ccg gtg acg gtg tcg tgg aac tca ggc 537
 Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly
 145 150 155 160

gct ctg acc agc ggc gtg cac acc ttc cca gct gtc cta cag tcc tca 585
 Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser
 165 170 175

gga ctc tac tcc ctc agc agc gtg gtg acc gtg ccc tcc agc aac ttc 633
 Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Asn Phe
 180 185 190

ggc acc cag acc tac acc tgc aac gta gat cac aag ccc agc aac acc 681
 Gly Thr Gln Thr Tyr Thr Cys Asn Val Asp His Lys Pro Ser Asn Thr
 195 200 205

aag gtg gac aag aca gtt gag cgc aaa tgt tgt gtc gag tgc cca ccg 729
Lys Val Asp Lys Thr Val Glu Arg Lys Cys Cys Val Glu Cys Pro Pro
210 215 220

tgc cca gca cca cct gtg gca gga ccg tca gtc ttc ctc ttc ccc cca 777
Cys Pro Ala Pro Pro Val Ala Gly Pro Ser Val Phe Leu Phe Pro Pro
225 230 235 240

aaa ccc aag gac acc ctc atg atc tcc cgg acc cct gag gtc acg tgc 825
Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys
245 250 255

gtg gtg gtg gac gtg agc cac gaa gac ccc gag gtc cag ttc aac tgg 873
Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Gln Phe Asn Trp
260 265 270

tac gtg gac ggc gtg gag gtg cat aat gcc aag aca aag cca cgg gag 921
Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu
275 280 285

gag cag ttc aac agc acg ttc cgt gtg gtc agc gtc ctc acc gtt gtg 969
Glu Gln Phe Asn Ser Thr Phe Arg Val Val Ser Val Leu Thr Val Val
290 295 300

cac cag gac tgg ctg aac ggc aag gag tac aag tgc aag gtc tcc aac 1017
His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn
305 310 315 320

aaa ggc ctc cca gcc ccc atc gag aaa acc atc tcc aaa acc aaa ggg 1065
Lys Gly Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Thr Lys Gly
325 330 335

cag ccc cga gaa cca cag gtg tac acc ctg ccc cca tcc cgg gag gag 1113
Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu
340 345 350

21

atg acc aag aac cag gtc agc ctg acc tgc ctg gtc aaa ggc ttc tac 1161
 Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr
 355 360 365

ccc agc gac atc gcc gtg gag tgg gag agc aat ggg cag ccg gag aac 1209
 Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn
 370 375 380

aac tac aag acc aca cct ccc atg ctg gac tcc gac ggc tcc ttc ttc 1257
 Asn Tyr Lys Thr Thr Pro Pro Met Leu Asp Ser Asp Gly Ser Phe Phe
 385 390 395 400

ctc tac agc aag ctc acc gtg gac aag agc agg tgg cag cag ggg aac 1305
 Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn
 405 410 415

gtc ttc tca tgc tcc gtg atg cat gag gct ctg cac aac cac tac aca 1353
 Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr
 420 425 430

cag aag agc ctc tgc ctg tct ctg ggt aaa tga gaattc 1392
 Gln Lys Ser Leu Cys Leu Ser Leu Gly Lys
 435 440

<210> 9

<211> 442

<212> PRT

<213> Artificial Sequence

<223> Description of Artificial Sequence: Synthetic
 sequence

<400> 9

Gln Ile Gln Leu Val Gln Ser Gly Pro Glu Leu Lys Lys Pro Gly Glu
 1 5 10 15

Thr Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asn Tyr
 20 25 30

22

Gly Met Asn Trp Val Arg Gln Ala Ser Gly Glu Gly Leu Lys Trp Met
 35 40 45

Gly Trp Ile Asn Thr Tyr Thr Gly Glu Pro Thr Tyr Gly Glu Asp Phe
 50 55 60

Lys Gly Arg Phe Ala Phe Ser Leu Glu Thr Ser Ala Ser Thr Ala Tyr
 65 70 75 80

Leu Gln Ile Asn Asn Leu Lys Asn Glu Asp Thr Ala Thr Tyr Phe Cys
 85 90 95

Ala Arg Phe Gly Asn Tyr Val Asp Tyr Trp Gly Gln Gly Thr Thr Leu
 100 105 110

Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala
 115 120 125

Pro Cys Ser Arg Ser Thr Ser Glu Ser Thr Ala Ala Leu Gly Cys Leu
 130 135 140

Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly
 145 150 155 160

Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser
 165 170 175

Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Asn Phe
 180 185 190

Gly Thr Gln Thr Tyr Thr Cys Asn Val Asp His Lys Pro Ser Asn Thr
 195 200 205

Lys Val Asp Lys Thr Val Glu Arg Lys Cys Cys Val Glu Cys Pro Pro
 210 215 220

Cys Pro Ala Pro Pro Val Ala Gly Pro Ser Val Phe Leu Phe Pro Pro
 225 230 235 240

23

Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys
245 250 255

Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Gln Phe Asn Trp
260 265 270

Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu
275 280 285

Glu Gln Phe Asn Ser Thr Phe Arg Val Val Ser Val Leu Thr Val Val
290 295 300

His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn
305 310 315 320

Lys Gly Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Thr Lys Gly
325 330 335

Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu
340 345 350

Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr
355 360 365

Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn
370 375 380

Asn Tyr Lys Thr Thr Pro Pro Met Leu Asp Ser Asp Gly Ser Phe Phe
385 390 395 400

Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn
405 410 415

Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr
420 425 430

Gln Lys Ser Leu Cys Leu Ser Leu Gly Lys
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<211> 1392

<212> DNA

<213> Artificial Sequence

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<223> Description of Artificial Sequence: Synthetic
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<210> 11

<211> 238

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic
sequence

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Val His Ser Asp Ile Val Met Thr Gln Ser Pro Leu Ser Leu Pro Val
20 25 30

Thr Pro Gly Glu Pro Ala Ser Ile Ser Cys Arg Ser Ser Lys Asn Leu
35 40 45

Leu His Ser Asn Gly Ile Thr Tyr Leu Tyr Trp Tyr Leu Gln Lys Pro
50 55 60

Gly Gln Ser Pro Gln Leu Leu Ile Tyr Gln Met Ser Asn Leu Ala Ser
65 70 75 80

Gly Val Pro Asp Arg Phe Ser Ser Ser Gly Ser Gly Thr Asp Phe Thr
85 90 95

Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys
100 105 110

Ala Gln Asn Leu Glu Ile Pro Arg Thr Phe Gly Gln Gly Thr Lys Val
115 120 125

Glu Ile Lys Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro
130 135 140

26

Ser Asp Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu
145 150 155 160

Asn Asn Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn
165 170 175

Ala Leu Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser
180 185 190

Lys Asp Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala
195 200 205

Asp Tyr Glu Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly
210 215 220

Leu Ser Ser Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
225 230 235

<210> 12

<211> 465

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic
sequence

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1 5 10 15

Val His Ser Gln Val Gln Leu Val Gln Ser Gly Pro Glu Val Lys Lys
20 25 30

Pro Gly Ala Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe
35 40 45

27

Thr Asn Tyr Gly Met Asn Trp Val Arg Gln Ala Pro Gly Gln Gly Leu
 50 55 60

Glu Trp Met Gly Trp Ile Asn Thr Tyr Thr Gly Glu Pro Thr Tyr Gly
 65 70 75 80

Glu Asp Phe Lys Gly Arg Phe Ala Phe Ser Leu Asp Thr Ser Ala Ser
 85 90 95

Thr Ala Tyr Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val
 100 105 110

Tyr Phe Cys Ala Arg Phe Gly Asn Tyr Val Asp Tyr Trp Gly Gln Gly
 115 120 125

Ser Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe
 130 135 140

Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu
 145 150 155 160

Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp
 165 170 175

Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu
 180 185 190

Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser
 195 200 205

Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro
 210 215 220

Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys
 225 230 235 240

Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro
 245 250 255

28

Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser
260 265 270

Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp
275 280 285

Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn
290 295 300

Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val
305 310 315 320

Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu
325 330 335

Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys
340 345 350

Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr
355 360 365

Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr
370 375 380

Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu
385 390 395 400

Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu
405 410 415

Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys
420 425 430

Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu
435 440 445

Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly
450 455 460

Lys

465

<210> 13

<211> 238

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic
sequence

<400> 13

Met Gly Trp Ser Cys Ile Ile Leu Phe Leu Val Ala Thr Ala Thr Gly
1 5 10 15

Val His Ser Asp Ile Val Met Thr Gln Ser Pro Leu Ser Leu Pro Val
20 25 30

Thr Pro Gly Glu Pro Ala Ser Ile Ser Cys Arg Ser Ser Lys Asn Leu
35 40 45

Leu His Ser Asn Gly Ile Thr Tyr Leu Tyr Trp Tyr Leu Gln Lys Pro
50 55 60

Gly Gln Ser Pro Gln Leu Leu Ile Tyr Gln Met Ser Asn Leu Ala Ser
65 70 75 80

Gly Val Pro Asp Arg Phe Ser Ser Ser Gly Ser Gly Thr Asp Phe Thr
85 90 95

Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys
100 105 110

Ala Gln Asn Leu Glu Ile Pro Arg Thr Phe Gly Gln Gly Thr Lys Val
115 120 125

30

Glu Ile Lys Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro
130 135 140

Ser Asp Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu
145 150 155 160

Asn Asn Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn
165 170 175

Ala Leu Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser
180 185 190

Lys Asp Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala
195 200 205

Asp Tyr Glu Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly
210 215 220

Leu Ser Ser Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
225 230 235

<210> 14

<211> 462

<212> PRT

<213> Artificial Sequence

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<223> Description of Artificial Sequence: Synthetic
sequence

<400> 14

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1 5 10 15

Val His Ser Gln Val Gln Leu Val Gln Ser Gly Pro Glu Val Lys Lys
20 25 30

Pro Gly Ala Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe
35 40 45

Thr Asn Tyr Gly Met Asn Trp Val Arg Gln Ala Pro Gly Gln Gly Leu
50 55 60

Glu Trp Met Gly Trp Ile Asn Thr Tyr Thr Gly Glu Pro Thr Tyr Gly
65 70 75 80

Glu Asp Phe Lys Gly Arg Phe Ala Phe Ser Leu Asp Thr Ser Ala Ser
85 90 95

Thr Ala Tyr Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val
100 105 110

Tyr Phe Cys Ala Arg Phe Gly Asn Tyr Val Asp Tyr Trp Gly Gln Gly
115 120 125

Ser Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe
130 135 140

Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser Glu Ser Thr Ala Ala Leu
145 150 155 160

Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp
165 170 175

Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu
180 185 190

Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser
195 200 205

Ser Ser Leu Gly Thr Lys Thr Tyr Thr Cys Asn Val Asp His Lys Pro
210 215 220

Ser Asn Thr Lys Val Asp Lys Arg Val Glu Ser Lys Tyr Gly Pro Pro
225 230 235 240

32

Cys Pro Pro Cys Pro Ala Pro Glu Phe Ala Gly Ala Pro Ser Val Phe
 245 250 255

Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro
 260 265 270

Glu Val Thr Cys Val Val Val Asp Val Ser Gln Glu Asp Pro Glu Val
 275 280 285

Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr
 290 295 300

Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Tyr Arg Val Val Ser Val
 305 310 315 320

Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Ala Tyr Lys Cys
 325 330 335

Lys Val Ser Asn Lys Gly Leu Pro Ser Ser Ile Glu Lys Thr Ile Ser
 340 345 350

Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro
 355 360 365

Ser Gln Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val
 370 375 380

Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly
 385 390 395 400

Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp
 405 410 415

Gly Ser Phe Phe Leu Tyr Ser Arg Leu Thr Val Asp Lys Ser Arg Trp
 420 425 430

Gln Glu Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His
 435 440 445

Asn His Tyr Thr Gln Lys Ser Leu Cys Leu Ser Leu Gly Lys

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460

<210> 15

<211> 238

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic
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<400> 15

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Val His Ser Asp Ile Val Met Thr Gln Ser Pro Leu Ser Leu Pro Val

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Thr Pro Gly Glu Pro Ala Ser Ile Ser Cys Arg Ser Ser Lys Asn Leu

35

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45

Leu His Ser Asn Gly Ile Thr Tyr Leu Tyr Trp Tyr Leu Gln Lys Pro

50

55

60

Gly Gln Ser Pro Gln Leu Leu Ile Tyr Gln Met Ser Asn Leu Ala Ser

65

70

75

80

Gly Val Pro Asp Arg Phe Ser Ser Ser Gly Ser Gly Thr Asp Phe Thr

85

90

95

Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys

100

105

110

Ala Gln Asn Leu Glu Ile Pro Arg Thr Phe Gly Gln Gly Thr Lys Val

115

120

125

34

Glu Ile Lys Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro
 130 135 140

Ser Asp Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu
 145 150 155 160

Asn Asn Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn
 165 170 175

Ala Leu Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser
 180 185 190

Lys Asp Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala
 195 200 205

Asp Tyr Glu Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly
 210 215 220

Leu Ser Ser Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
 225 230 235

<210> 16

<211> 461

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic
 sequence

<400> 16

Met Gly Trp Ser Cys Ile Ile Leu Phe Leu Val Ala Thr Ala Thr Gly
 1 5 10 15

Val His Ser Gln Val Gln Leu Val Gln Ser Gly Pro Glu Val Lys Lys
 20 25 30

35

Pro Gly Ala Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe
 35 40 45

Thr Asn Tyr Gly Met Asn Trp Val Arg Gln Ala Pro Gly Gln Gly Leu
 50 55 60

Glu Trp Met Gly Trp Ile Asn Thr Tyr Thr Gly Glu Pro Thr Tyr Gly
 65 70 75 80

Glu Asp Phe Lys Gly Arg Phe Ala Phe Ser Leu Asp Thr Ser Ala Ser
 85 90 95

Thr Ala Tyr Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val
 100 105 110

Tyr Phe Cys Ala Arg Phe Gly Asn Tyr Val Asp Tyr Trp Gly Gln Gly
 115 120 125

Ser Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe
 130 135 140

Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser Glu Ser Thr Ala Ala Leu
 145 150 155 160

Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp
 165 170 175

Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu
 180 185 190

Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser
 195 200 205

Ser Asn Phe Gly Thr Gln Thr Tyr Thr Cys Asn Val Asp His Lys Pro
 210 215 220

Ser Asn Thr Lys Val Asp Lys Thr Val Glu Arg Lys Cys Cys Val Glu
 225 230 235 240

36

Cys Pro Pro Cys Pro Ala Pro Pro Val Ala Gly Pro Ser Val Phe Leu
245 250 255

Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu
260 265 270

Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Gln
275 280 285

Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys
290 295 300

Pro Arg Glu Glu Gln Phe Asn Ser Thr Phe Arg Val Val Ser Val Leu
305 310 315 320

Thr Val Val His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys
325 330 335

Val Ser Asn Lys Gly Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys
340 345 350

Thr Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser
355 360 365

Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys
370 375 380

Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln
385 390 395 400

Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Met Leu Asp Ser Asp Gly
405 410 415

Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln
420 425 430

Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn
435 440 445

His Tyr Thr Gln Lys Ser Leu Cys Leu Ser Leu Gly Lys

450

455

460

INTERNATIONAL SEARCH REPORT

International Application No

PCT/EP 99/05271

A. CLASSIFICATION OF SUBJECT MATTER
 IPC 7 A61K39/395 //A61K38:16, A61K31:00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	PAUL A R ET AL: "Treatment of advanced measurable or evaluable pancreatic carcinoma with 17-1A murine monoclonal antibody alone or in combination with 5-fluorouracil, adriamycin and mitomycin (FAM)." HYBRIDOMA, (1986 JUL) 5 SUPPL 1 S171-4. , XP000881980 the whole document.	1-15

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents:

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

- *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- *Z* document member of the same patent family

Date of the actual completion of the international search

20 March 2000

Date of mailing of the international search report

14. 04. 00

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
 NL - 2280 HV Rijswijk
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Mennessier, T

INTERNATIONAL SEARCH REPORT

nal Application No

PCT/EP 99/05271

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	KIEVIT E ET AL: "Determination of tumor-related factors of influence on the uptake of the monoclonal antibody 323/A3 in experimental human ovarian cancer." INTERNATIONAL JOURNAL OF CANCER, (1997 APR 10) 71 (2) 237-45. , XP000882050 page 238, right-hand column -page 240, left-hand column page 243; table III ---	1-15
Y	BOKEMEYER C ET AL: "Current aspects of adjuvant and palliative chemotherapy in colorectal carcinoma!. Aktuelle Aspekte zur adjuvanten und palliativen Chemotherapie beim kolorektalen Karzinom." SCHWEIZERISCHE RUNDSCHAU FUR MEDIZIN PRAXIS, (1997 SEP 24) 86 (39) 1510-6 REF: 11 , XP000882022 page 1515, paragraph 3.4; table 8 ---	1-15
Y	CASILLAS S ET AL: "Adjuvant therapy for colorectal cancer: present and future perspectives." DISEASES OF THE COLON AND RECTUM, (1997 AUG) 40 (8) 977-92. REF: 80 , XP000882030 page 989, left-hand column page 980; table 1 ---	1-15
Y	EP 0 252 741 A (CENTOCOR INC) 13 January 1988 (1988-01-13) page 2, line 58-63 page 3, line 8-13 ---	1-15
A	BLEIBERG H: "Continuing the fight against advanced colorectal cancer: new and future treatment options." ANTI-CANCER DRUGS, (1998 JAN) 9 (1) 18-28. REF: 83 , XP000882025 page 23 page 24; table 1 ---	1-15
A	ELIAS D J ET AL: "Monoclonal antibody KS1/4-methotrexate immunoconjugate studies in non-small cell lung carcinoma." AMERICAN JOURNAL OF RESPIRATORY AND CRITICAL CARE MEDICINE, (1994 OCT) 15 (4) 1114-22. , XP000882026 page 1114 page 1121, left-hand column ---	1-15

-/--

INTERNATIONAL SEARCH REPORT

International Application No

PCT/EP 99/05271

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	HAISMA H J ET AL: "A monoclonal antibody-beta-glucuronidase conjugate as activator of the prodrug epirubicin-glucuronide for specific treatment of cancer." BRITISH JOURNAL OF CANCER, (1992 SEP) 66 (3) 474-8. , XP000882039 the whole document	1-15
P,X	SCHWARTZBERG, LEE S. (1): "Chemotherapy plus PANOREX (17-1A monoclonal antibody) as adjuvant therapy for colon cancer: Ongoing studies." CANCER INVESTIGATION, (1999) VOL. 17, NO. SUPPL. 1, PP. 32-34. MEETING INFO.: XVI CHEMOTHERAPY FOUNDATION SYMPOSIUM ON INNOVATIVE CANCER THERAPY FOR TOMORROW NEW YORK CITY, NEW YORK, USA NOVEMBER 11-13, 1998 CHEMOTHERAPY FOUNDATION. , XP000882015 the whole document	1-15

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP 99/05271

Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 12-14 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the co-administered chemotherapeutic agent and anti-Ep-CAM antibody.
2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/EP 99/05271

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 0252741 A	13-01-1988	AT 159174 T	15-11-1997
		DE 3752129 D	20-11-1997
		DE 3752129 T	07-05-1998
		EP 0755683 A	29-01-1997
		ES 2110392 T	16-02-1998
		GR 3025902 T	30-04-1998
		HK 1002829 A	18-09-1998
		JP 2979318 B	15-11-1999
		JP 63060941 A	17-03-1988
		JP 2000026312 A	25-01-2000
